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THE EFFECT OF ACCLIMATION TEMPERATURE ON OXYGEN CONSUMPTION AND BLOOD PROPERTIES OF LARVAL ARCTIC LAMPREY, *LAMPETRA JAPONICA*

by

© SANDRA C. BOURQUE

A THESIS

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Effect of Acclimation Temperature on Oxygen Consumption and Blood Properties of Larval Arctic Lamprey, *Lampetra japonica*" submitted by Sandra C. Bourque in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

Low routine rates of oxygen consumption of larval Arctic lamprey held several months at 18, 10 and 2C under a natural photoperiod were measured at the respective acclimation temperatures in January, March, and May, and at various other temperatures over the range of 2 to 24C in May. Periodic measurements of oxygen consumption and weight were taken for marked ammocoetes at 2 and 10C from January to August. Hematocrits, hemoglobin concentrations, and oxygen equilibria were measured in September from blood of ammocoetes acclimated at 2 and 10C.

1. The average rate of oxygen consumption of ammocoetes acclimated at 2C - 0.028 mg/g/hr - was significantly lower than the average for 18C acclimated animals - 0.043 mg/g/hr.

2. Oxygen consumption and weight of marked ammocoetes varied seasonally at 10C, but was stable at 2C. Oxygen consumption of 10C acclimated animals was 0.040 mg/g/hr in January and March, decreased to 0.030 mg/g/hr in May, then increased to 0.068 mg/g/hr by the end of August.

3. The acclimation ability of ammocoetes is high - "long term" Q_{10} was 1.3 between 2 and 18C and compensation ranged from 60 to 90%.

4. "Acute" Q_{10} values of 1.77, 2.18, and 2.82 for animals acclimated at 2, 10, and 18C respectively also indicate that ammocoetes had made significant metabolic adjustments at each acclimation temperature.

5. When expressed on a logarithmic grid, the slope of the regression relating body weight to oxygen uptake increased as acclimation temperature decreased. Thus a change in acclimation temperature has a greater effect on smaller ammocoetes. Regression slopes varied seasonally at each temperature, decreasing significantly between winter (January-March) and May.

6. Weight specific rates of oxygen uptake were not weight dependent except in May at 18 and 10C.

7. There was no significant difference in hematocrits, hemoglobin concentration or oxygen equilibria curves between animals acclimated at 2 and 10C. Average hematocrit was 34%, and P_{50}, 1.5 mmHg at both temperatures, while hemoglobin concentrations averaged 9.0 and 9.7 g% at 2 and 10C respectively.

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INTRODUCTION

Larval lamprey represent the most primitive level of vertebrate development. Consequently, their ability to adapt to environmental changes should be of interest to those studying the adaptive abilities of more advanced vertebrates. However, at the time this study was initiated, little information was available on cyclostomes. The main objective of this study, therefore, was to determine the ability of larval Arctic lamprey, *Lampetra japonica* (Martens), to adapt to temperature change.

Arctic lamprey used in this study spend four to six years as larvae (or ammocoetes) in the Hay River, Northwest Territories, and an unknown length of time as parasitic adults in Great Slave Lake (Buchwald, 1968). Larvae of all species spend most of their time burrowed along the margins of streams and rivers but they can be forced out of the mud by near upper lethal temperatures (McCauley, 1962) and low oxygen tensions (Potter, Hill and Gentleman, 1970). Water temperatures in the Hay River range from 0C through late October to early May to 22C in the summer months. In winter, water temperatures are stable, but in summer, daily temperature changes average 1C and fluctuations of 4C are not uncommon, especially in the shallow shore and backwater areas which larvae prefer. Maximum temperatures experienced by larvae in these shallow areas have not been recorded.

Although the body temperature of a poikilotherm normally conforms to such seasonal and daily temperature changes, metabolic rate is commonly regulated to some extent (Rao and Bullock, 1954; Precht, 1958; Prosser and Brown, 1961; Fry, 1971; Vernberg and Vernberg, 1970). Maintenance of rate functions at similar levels with changing external temperature is defined as thermal acclimation and is widespread in poikilotherms. Acclimation allows poikilotherms to avoid the disruptive and potentially lethal effects of temperature change.

Changes in body temperature alter the amount of energy available to molecules thereby altering a) the rate at which covalent bonds of metabolic chemical reactions are broken and reformed and b) the stability of weak bonds which maintain the structural and therefore functional integrity of "higher order" biochemicals (e.g., 3° and 4° structure of structural proteins, enzymes, hormones, lipids, nucleic acids) (Hochachka and Somero, 1973). The magnitude of rate change in an organism's metabolic rate, as indicated by changes in oxygen consumption, is initially a two- to four-fold increase with each 10C° increase in temperature (Morris, 1965; Hoar, 1966; Peterson and Anderson, 1969; Fry and Hochachka, 1970). However, because metabolic rates must be maintained within certain limits for survival, temperature induced changes in the metabolic rate could be potentially lethal. Also, since the rate change of the individual reactions of metabolism is often greater than a two- to four-fold increase at physiological substrate concentrations, and the rate change varies between reactions (Baldwin, 1971; Moon and Hochachka, 1972; Newell and Pye, 1971), temperature changes could result in serious imbalances

between metabolic reactions (Hochachka and Somero, 1973). However, few deaths have been reported in the literature which can be attributed to the slow seasonal temperature changes such as occur in the temperate zones (Brett, 1956), and metabolic rates of animals held at different temperatures are often less than two times greater for each 10C° increase, although this varies with the activity, species, size of the animal, and the temperature range considered (Job, 1955; Beamish, 1964; Brett, 1964; Rao, 1968; O'Hara, 1968, Peterson and Anderson, 1969).

The molecular changes behind acclimation are thought to occur primarily through changes in the structures of the "higher order" molecules where structure depends on weak bonds and is easily altered by temperature change. Reviews of findings on temperature induced changes at the molecular level have been made by Prosser (1967), Somero (1969), Fry and Hochachka (1970), and Hochachka and Somero (1971 and 1973) and the details are not within the scope of this thesis.

The adaptive significance of acclimating to seasonal temperature extremes has been clearly illustrated by displacement of lethal temperature limits as shown for goldfish [*Carassius auratus*] (Fry, Brett, and Clawson, 1942), brown bullhead [*Ameiurus nebulosus* = *Ictalurus nebulosus*] (Brett, 1944), brook trout [*Salvelinus fontinalis*] (Fry, Hart and Walker, 1946), chum and sockeye salmon [*Oncorhynchus keta* and *O. nerka*] (Brett, 1952; Brett and Alderdice, 1958), sea lamprey [*Petromyzon marinus*] (McCauley, 1962), Mozambique cichlid [*Tilapia mossambica*] (Allanson and Noble, 1964), and others (Brett, 1956). McCauley (1962) has shown that the upper lethal temperature of larval sea lamprey can be increased from

27.7 to 31.7C with a change in acclimation temperature from 2 to 31C. This represents an average change of 0.9C° with each 5C° change in acclimation temperature, except between 10 and 15C when the upper lethal temperature increased 1.2C° and between 15 and 20C when the upper limit did not change significantly. McCauley (1962) also found that larvae are more temperature tolerant than adults, although if acclimated, both can survive indefinitely at temperatures as high as 25 and as low as 2C. The lower lethal temperature decreased sharply between 30 and 20C but remained relatively constant at 0-1C for animals acclimated at temperatures less than 20C. The 4C° extension in the upper lethal limit for sea lamprey is comparable to that of the common shiner [*Notropis cornutus*] (Hart, 1947), which is also found in shallow waters of northern streams where temperatures vary from warm to cool depending on the summer weather. Upper lethal temperatures for larval and adult sea lamprey are higher than those of cold water fish such as various species of Pacific salmon (Brett, 1952), and brook trout (Fry et al., 1946), however, they are not as high as those of warm water species such as the brown bullhead (Brett, 1944) and goldfish (Fry et al., 1942).

In addition to extending lethal limits, acclimation at near lethal temperatures increases the length of time that sea lamprey can resist lethal temperatures (McCauley, 1962) - an essential adaptation for survival in areas where temperatures pass beyond lethal limits for short periods of time. Similar results have been found for greenfish [*Girella nigricans*] (Doudoroff, 1942), goldfish (Fry et al., 1942), chum and sockeye salmon (Brett, 1952; Brett and Alderdice, 1958), northern

redbelly dace [*Chrosomus eos*] and finescale dace [*C. neogaeus*] (Tyler, 1966). Also, the resistance time at lethal temperatures is increased as the time span for the temperature change increases (Cocking, 1959; Tyler, 1966).

The adaptive significance of compensating for small temperature changes within the lethal limits is not as clearly defined. For larval lamprey, an inability to maintain a stable level of metabolic activity during summer months, regardless of slight temperature changes, would likely interfere with maximum utilization of seasonal food sources. Thus growth and development, and over several summers, perhaps even age of transformation could be affected by the ability of larvae to quickly compensate for temperature changes.

This is particularly important since Moore and Beamish (1973) have shown that injestion of algae and digestive ability are greatly reduced at normal winter temperatures (0C) in larval sea lamprey. Also, Hardisty (1961) has shown correlated increases in growth and fat storage in larval European brook lamprey [*Lampetra planeri*] with increases in the density of phytoplankton in spring, while field studies have shown that mountain brook lamprey [*Icthyomyzon hubbsi*] show no increase in length during the winter months when temperatures are low (Hill and Potter, 1970). Thus much of an ammocoete's growth is limited to a few months of warm weather when food supplies are abundant.

Few studies have been done to determine the time involved for poikilotherms to acclimate to small, rapid changes in temperature. Peterson and Anderson (1969) found that active oxygen consumption and

activity of juvenile Atlantic salmon [*Salmo salar*] whose acclimation temperature was changed from 6 to 9C or from 18 to 15C over thirty minutes, returned to their original levels within one hour of the change. When temperatures were changed 6C° or more, active metabolic rates did not return to the original levels, but the actual time required for complete acclimation was not determined. Acclimation to temperature changes has required days or weeks for brook trout (Brett, 1941), immature greenfish (Doudoroff, 1942), and goldfish (Fry and Hart, 1948; Kanugo and Prosser, 1959), and the rate of acclimation apparently depends on the rate of temperature change (Cocking, 1959).

Immediate and complete temperature compensation have been measured for the winkle [*Littorina littorea*], which is exposed daily to large (20C° maximum) rapid changes in body temperature (Newell and Pye, 1970). However, the temperature independence of oxygen consumption was only evident in May when air temperatures were 15C, but not in January when air temperatures were 4C. Complete temperature compensation of oxygen consumption has also been observed for sunfish [*Lepomis gibbosus*] acclimated between 10 and 17.5C, although the time required for complete compensation was not determined (Roberts, 1967).

Acclimation ability has most often been determined by comparing the metabolic rates of animals which have been held for extended periods of time at different, constant "acclimation" temperatures, or by comparing the metabolic rates of animals held at different acclimation temperatures but measured at various temperatures. Morris (1962) has defined the first type of measurement as "long-term" and the second as "acute."

Classification schemes to define the degree of acclimation have been designed by Precht (1958) for the "long-term" method and by Prosser (1961) for the "acute" method. Oxygen consumption has most frequently been used as the measure of metabolic rate.

Using the "acute" method, Sherbakov (1937) found some degree of acclimation in adult river lamprey [*Lampetra fluviatilis*] since as holding temperature decreased from 17 to 1.5C, oxygen consumption increased from 0.14 to 0.21 mg/g/hr when measured at approximately 16C. Calculations of Q_{10} from "long-term" measurements of oxygen consumption (i.e. measured at the acclimation temperature) show that adult sea lamprey adapt well to different temperatures as the Q_{10} was 1.8 between 5 and 20C (Beamish, 1973), while larval mountain brook lamprey have a poor ability to compensate for temperature changes since Q_{10} was 3.6 between 3.5 and 22.5C (Hill and Potter, 1970). A Q_{10} of 1.6 calculated for adult Pacific lamprey [*Entosphenus tridentatus*] acclimated at 14-16C tested over the range of 5 to 20C (Johansen, Lenfant and Hanson, 1973) suggests that this species may be able to compensate rapidly for temperature change, however the exact exposure time at each test temperature was not given.

This thesis presents the results of experiments to determine the rates of oxygen consumption of larval Arctic lamprey held several months at 18, 10 and 2C measured both at the temperature of acclimation and at various other temperatures over the range of 2 to 24C. Because measurements were taken over several months and the acclimation ability of some fish held at constant temperatures is known to vary seasonally (Hart,

1952; Hoar and Robertson, 1959; Tyler, 1966), one experiment was devoted to determining if ammocoetes acclimated at a constant temperature exhibited seasonal changes in metabolic rate. Also, since body size has been shown to influence "acutely" measured Q_{10} values (Rao and Bullock, 1954), and there are indications that as acclimation temperature increases oxygen consumption of large fish may not increase to the same relative extent as for small fish (Job, 1955; Beamish, 1964; O'Hara, 1968), all results presented here have been analysed for the effect of body weight on the relationship between oxygen consumption and acclimation or test temperature.

Hill and Potter (1970) demonstrated that oxygen consumption per unit weight of larval mountain brook lamprey decreased with increasing size, however in adult sea lamprey oxygen uptake per unit weight was not related to body size (Beamish, 1973). The increase in oxygen uptake with acclimation temperature was independent of size for adult sea lamprey (Beamish, 1973) but this relationship has not been measured for ammocoetes.

Also presented are measurements of blood hemoglobin content, hematocrit, and oxygen equilibria taken to determine if the oxygen carrying ability of blood is altered when lamprey are acclimated at different temperatures for long periods of time. De Wilde and Houston (1967) have suggested that an increase in the blood oxygen carrying capacity with an increase in temperature could be the least costly of the cardiovascular-respiratory adjustments which could be made to accommodate the increased oxygen demand.

Erythrocyte count, hematocrit, hemoglobin concentration, and hemoglobin content of erythrocytes were higher in rainbow trout [*Salmo gairdneri*] acclimated at 11, 14 and 17C than in trout acclimated at 3 and 7C; while trout at 21C had significantly larger hemoglobin concentrations and numbers of slightly smaller erythrocytes which contained a little more hemoglobin per erythrocyte than animals at 11, 14 and 17C or at 3 and 7C (De Wilde and Houston, 1967). The greatest difference between hematological measurements of trout acclimated at different temperatures occurred in fall. However, Cameron (1971) was unable to demonstrate any difference in the oxygen dissociation curves of blood from rainbow trout acclimated at 18 and 7-9C. Grigg (1969) had reported a shift in the P_{50} with acclimation temperature in brown bullhead. In the carp [*Cyprinus carpio*], erythrocyte count, hematocrit, and hemoglobin concentration, but not erythrocyte hemoglobin content increased with acclimation temperature, but the difference was not significant except in fall when values for 27 and 33C acclimated animals were significantly higher than those of 2 and 4C acclimated carp (Houston and De Wilde, 1968). At the lowest acclimation temperatures (2, 4, 7C), these values increased between summer and winter. Black, Kirkpatric, and Tucker (1966a,b,c) did not find any significant difference in oxygen capacity, red cell volume or oxygen equilibria of brook trout, Atlantic salmon or landlocked Atlantic salmon [*S. salar sebago*] acclimated at 5 and 20C. The hematology of goldfish at different temperatures has also been extensively studied but no consistent pattern of change with temperature has been observed (see Houston and De Wilde (1968) for a review of results on goldfish).

Although the blood characteristics of lamprey have been extensively researched, little work has been done comparing the blood of animals acclimated at different temperatures. The only such investigation showed that the pH of blood from larval mountain brook lamprey acclimated at 5, 15.5 and 22.5C decreased with increasing temperature (Potter et al., 1970).

MATERIALS AND METHODS

A. Source and Maintenance of Animals

Larval *Lampetra japonica* were collected with a hand operated AC electroshocker[1] from the Hay River near its confluence with Great Slave Lake (115°49'W, 60°45'N) in August 1968. Most of the larvae were found in fine gravel covered with a thin layer of silt seven miles upstream from the mouth of the river, however the largest animals (>8 gm) were found in deep mud banks nearer the lake. Larvae were never found in areas where bubbles of gas rose from the mud.

Animals were transported to Edmonton in 36 liter plastic garbage cans containing a layer of sediment, and river water which was periodically aerated with compressed oxygen. Water temperatures were initially 11C and increased to 13C by the end of the trip.

In the laboratory, ammocoetes were kept in 30 or 36 liter plastic containers supplied with natural sediments and aerated, dechlorinated tap water. Each container held a maximum of 30 animals. Approximately five animals 80-100 mm long, 30 animals 100-150 mm long and 40 animals 150-200 mm long were maintained at each of three acclimation temperatures: 18.0 $\pm$ 0.5, 10.0 $\pm$ 0.2 and 2.0 $\pm$ 0.2C.

---

[1] Portable 1750 watt, 110 volt AC generator; two electrode paddles, one equipped with an on-off microswitch (Buchwald, 1968).

Containers of animals to be maintained at 10 and 18C were placed in two 720 liter tanks of dechlorinated tap water. The walls of the containers were perforated to allow tank water to enter. Fresh water flow into the tanks was kept minimal to insure adequate temperature regulation. Temperature controlled refrigeration units were used to maintain water at 10C in one tank throughout the year and at 18C in the other tank during the summer. A temperature controlled heater was used to maintain water at 18C during the winter.

Containers of animals to be kept at 2C were placed in a constant temperature room. There was no water flow through these containers but the water was well aerated and changed once every three weeks. Ammonia concentration[2] in the containers never exceeded 0.05 mg/l.

All animals were held at 15C for two weeks after capture. Water temperatures were then changed 1C a day until the appropriate acclimation temperatures were reached. An eight week exposure to the acclimation temperature was allowed before measurements of oxygen consumption were taken. Photoperiod was maintained to that of the Edmonton latitude.

Although ammocoetes were initially fed a commercial fish food and an algal solution[3] twice a week, weights of marked individuals declined sharply from October to December 1968(Figure 4). A preliminary experiment showed that animals which were fed powdered brine shrimp maintained or gained weight, while those fed algae or powdered fish meal lost weight. Thus after December 1968, ammocoetes held at 10 and 18C

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[2]Determined colorimetrically with Nessler reagent (Fischer, 1964).

[3]Consisting primarily of the diatoms *Asterionella formosa*, *Fragilaria spp.*, *Scenedesmus quadricauda* and *Navicula spp.*

were fed powdered brine shrimp twice a week, and those at 2C were fed once a week.

B. Determination of Oxygen Consumption

1. Apparatus for measuring oxygen consumption

The apparatus used to measure oxygen consumption consisted of a reservoir of aerated water, respiration chambers (Figure 1a), and a temperature controlled water bath. The respiration chambers were Pyrex tubes 2.5 cm in diamenter, 8 to 20 cm long, and the length of tube used depended on the size of the animal. Chamber volumes varied from 14 to 105 ml of water when sealed with rubber stoppers. One stopper was fitted with a polyethylene tube (3 mm internal diameter) extending into the chambers for two thirds of its length, and a 14 G Luer-lock syringe needle. The polyethylene tube was connected to a 20 liter reservoir by pliable rubber tubing and water flow into the chamber was regulated by a Hoffman clamp on the rubber tubing. During determinations of oxygen consumption, the polyethylene tube was sealed by a pinch clamp and disconnected from the rubber tubing.

The syringe needle served for water outflow and collection of water samples for oxygen analysis. During determinations of oxygen consumption, the needle was fitted with a 10 cc Luer-lock syringe used to collect water samples (Figure 1a).

Respiration chambers were held vertically in weighted wooden holders and submerged in a Fischer Isotherm refrigerated bath[4] (Figure 1b).

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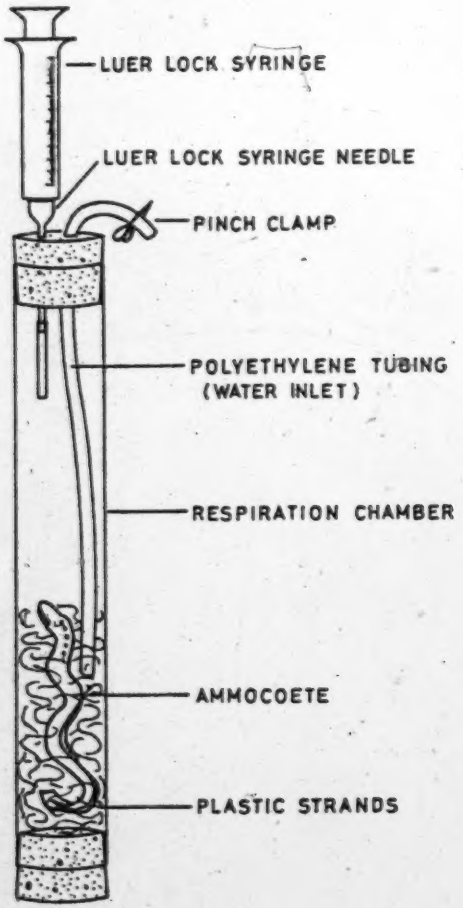
[4]Fischer Scientific Company.

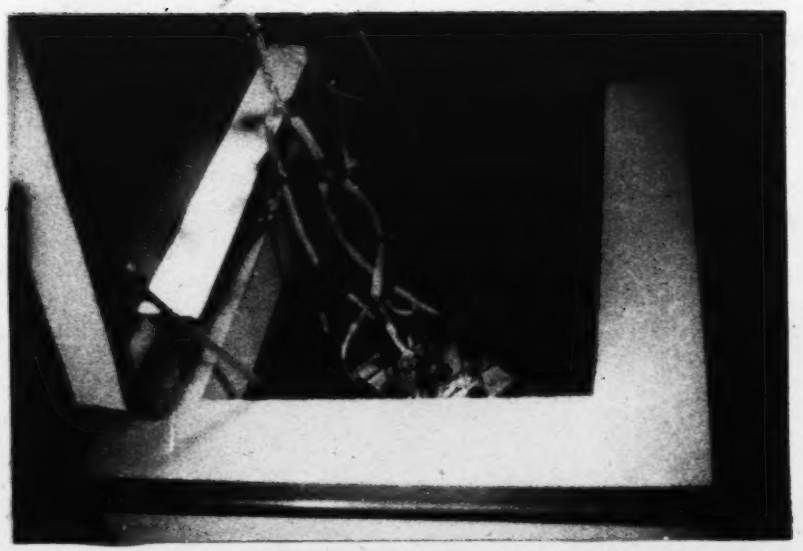
Figure 1. Apparatus used for measuring oxygen consumption.

a) A single respiration chamber as it would appear during measurement of oxygen consumption.

b) Respiration chambers and holders in the temperature controlled water bath.


LUER LOCK SYRINGE
LUER LOCK SYRINGE NEEDLE
PINCH CLAMP
POLYETHYLENE TUBING
(WATER INLET)
RESPIRATION CHAMBER
AMMOCOETE
PLASTIC STRANDS

a)



b)

The water temperature of the bath was held at the required temperature ± 0.25C by a thermo-regulator.[5] Two holders, each containing six respiration chambers, were used in an experiment. In this way, oxygen consumption could be determined simultaneously for eleven animals, one chamber serving as a control.

2. Experimental method

a) Measurement of oxygen consumption at the temperature of acclimation.

Ammocoetes were dug from the mud and placed in clean water 24 hours before the start of an experiment. In water with no substrate, ammocoetes constantly attempted to burrow into the container bottom. Netting, bricks and strands of plastic from an unwound pot scrubber provided sufficient tactile stimuli and hiding places to prevent this response and quiet the animals. Strands of plastic were also used in the bottom of respiration chambers as a substrate substitute.

The morning of an experiment, ammocoetes were transferred to the chambers where they immediately wound themselves in the plastic strands, remaining inactive until prodded out at the end of the experiment. The chambers were stoppered, then placed in the bath and connected to the gravity feed reservoir. Bath and reservoir water were maintained at the acclimation temperature of the animals. The bath was covered with dark plastic, and the chambers flushed at the rate of 2 liters/hr for one hour.

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[5] YSI model 73, Yellow Springs Instrument Co. Inc., Yellow Springs, Ohio, U.S.A.

Although 17 hours were required for *I. hubbsi* larvae to reach a steady state of oxygen consumption (Hill and Potter, 1970), there was no significant difference between rates of oxygen consumption of *L. japonica* determined after one, two, or five hours in the chamber. Thus oxygen consumption was considered stable and measurements were begun after only one hour in the chamber.

At the end of the one hour stabilization period, a 10 cc water sample was taken from each chamber and the chambers were sealed and disconnected from the reservoir. After an hour test period, each chamber was gently shaken, the polyethylene tube opened, and a 10 cc water sample taken from the inverted chamber. During this procedure ammocoetes remained entwined in the plastic and rarely seemed disturbed.

After an experiment, the animals were anaesthetized in tricaine methane sulphonate (MS. 222, Sandoz), blotted dry, weighed, and measured for length. They were then returned to their original containers. Only one experiment using a maximum of 11 animals was conducted each day.

In this way, oxygen consumption was measured in January, March, and May 1969 for animals acclimated at 18, 10, and 2C. Although animals measured in January were not retested in March, some animals previously tested in January or March were retested in May. Since these ammocoetes could not be identified, all samples were treated as random samples and Student's t test (Steel and Torrie, 1960) was used to determine significant difference[6] between values recorded at different temperatures and times.

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[6]Except where otherwise stated the level of significance used for all data in this thesis was $P < 0.05$.

b) Measurement of oxygen consumption after short term exposure to temperatures other than the acclimation temperature.

The effect of short term exposure to temperatures other than the acclimation temperature on ammocoete oxygen consumption was determined in May 1969 for ammocoetes acclimated at 18, 10, and 2C. In each experiment, oxygen consumption of eleven animals was first determined at the acclimation temperature, as described previously, then at one of the other test temperatures: 2, 10, 18, or 24C. After the final water samples were taken from ammocoetes being tested at their acclimation temperature, the chambers were returned to the bath and connected to a reservoir of water at one of the other test temperatures. The bath was then drained and filled with water at this new temperature. Once the required temperature was reached in the chambers (5-10 min.), a 90 minute stabilization period was allowed before the chambers were closed for determinations of oxygen consumption. The test period varied from 10 minutes to an hour so that utilization of oxygen never exceeded 35%. Water samples were again taken at the end of the stabilization and test periods.

Each animal was tested at only one temperature other than the acclimation temperature and only one experiment was conducted in a day. After an experiment, animals were anaesthetized and measured, then returned to their original containers.

3. Oxygen analysis and calculation of oxygen consumption

The oxygen content of water samples was determined by the modified Winkler method described by Burke (1962) with the following modifications. Samples taken in an ungreased syringe were titrated with a 0.005 N sodium thiosulfate solution from a 5 ml microburette. The indicator was a 0.05% solution of starch in glycerine. Rate of oxygen consumption was obtained by substitution in the following equation:

$$R = \frac{[k(t_1 - t_2)]\ V}{WT} \times 60$$

where R = rate of oxygen consumption mg/g/hr

$k = \frac{8000 \times N \text{ of sodium thiosulfate}}{\text{volume of water}}$

t_1 = titer of the water sample taken at the end of the stabilization period

t_2 = titer of the water sample taken at the end of the test period

V = volume of the chamber minus the volume of water displaced by the plastic strands and the animal

W = weight of the animal

T = duration of the test period (min.)

C. Measurement of Seasonal Changes in Metabolism

To determine if ammocoetes acclimated at a constant temperature exhibited seasonal changes in metabolism, periodic measurements of oxygen consumption and body weight and length were taken from marked lampreys between October 1968 and August 1969. Fifteen larvae at each acclimation temperature were marked with subcutaneous injections of cadmium or mercuric sulfide (Wigley, 1959), and kept in separate containers for easy accessibility.

Determinations of oxygen consumption made in 1968 were extremely variable as plastic strands were not used in the chambers. Thus these results are not included. Because of a malfunction in the heating unit, no data is available after May for animals acclimated at 18C. A paired t test (Steel and Torrie, 1960) was used to test significant difference between measurements made in different months.

D. Measurement of Hematocrit, Hemoglobin Concentration, and Oxygen Equilibria

Blood samples were collected in August and September, 1969 from ammocoetes acclimated at 2 and 10C. Ammocoetes were quickly anaesthetized, blotted dry, then cut in half immediately posterior to the heart. The cut surface of the head portion was blotted and blood which oozed from cut blood vessels was collected in capillary tubes and micropipettes for immediate analysis. Samples from different individuals were not pooled.

1. Hematocrit

Duplicate hematocrits were determined using commercially heparinized microcapillary tubes which were filled three quarters full, sealed with plastic clay and spun for 10 minutes at 10,000 rpm in a microhematocrit centrifuge.[7] The volume of packed erythrocytes was measured with a scaled reading card[8] and reported as per cent of whole blood. Duplicates were averaged but never varied more than 1%. Although some fish blood is reported to clot in commercially heparinized capillary tubes (Larsen and Snieszko, 1961; McKnight, 1966), ammocoete blood did not clot or hemolyze in these tubes.

2. Hemoglobin concentration

Micropipettes used to collect blood for determination of hemoglobin concentration and oxygen equilibria were first rinsed with a 10% heparin solution and dried. Hemoglobin concentration was determined by the cyanmethemoglobin method[9] using bovine hemoglobin solutions as standard. Transmittance was measured with a colorimeter.[10]

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[7]Model 31, Chicago Surgical and Electrical Co.

[8]Graphic Reader 30-5, Chicago Surgical and Electrical Co.

[9]As outlined in "Methods and Calibrations," Catalogue No. 33-29-40, a methods manual for the Bausch and Lomb Spectronic 20 Colorimeter.

[10]Spectronic 20, Bausch and Lomb.

3. Oxygen equilibria

Oxygen equilibria of hemoglobin solutions were determined by a method adapted from those described by Wald and Riggs (1951), Rossi-Fannelli and Antonini (1958), Manwell (1963), and Antonini et al. (1964). Erythrocytes were washed twice with 0.7% isotonic saline. Twenty-five microliters of the washed, packed erythrocytes were then hemolysed in one milliliter of distilled water for one hour at 0C. This hemolysate was centrifuged at 35,000 rpm for 30 minutes to eliminate suspended cellular debris. Two hundred microliters of the cleared hemoglobin solution were added to one milliliter of 0.15 M Na_2HPO_4-KH_2PO_4 buffer at a pH of 6.0, 6.49 or 7.13.

The buffered hemoglobin solution was contained in a microcuvette (1 cm light path) fitted with a three-way valve which could be attached to a manometer and suction pump. In this cuvette, the hemoglobin solution was evacuated at various partial pressures of air and the absorbance measured at 565nm in a recording spectrophotometer.[11] Recordings were made initially at atmospheric air pressure, then after complete evacuation, at progressively increasing partial pressures of air, after exposure to pure oxygen, and finally, after chemical reduction with sodium hydrosulphite. Measurements were made at room temperature of 22-24C. Calculations of the percent oxygenation at each partial pressure of oxygen were made on the assumption that the change in absorbance at any wavelength is linearly related to the total number of ferroheme groups oxygenated.

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[11] Cary, Model 14, recording spectrophotometer.

RESULTS

A. Effect of Acclimation Temperature on Oxygen Consumption

Average rates of oxygen uptake measured at the acclimation temperature in January, March, and May 1969 are given in Figure 2. Oxygen uptake of animals acclimated at 2 and 18C did not vary significantly between months, but rates for animals acclimated at 10C decreased significantly in May. This unexpected decrease was found for four different groups of 10C acclimated animals tested on four different days between May 13 and June 8 (Appendix Table 7).

Average rates of oxygen consumption for animals acclimated at 18C were significantly higher than those of 2C acclimated animals each month measured. In January and March, average rates of oxygen consumption of 10C acclimated animals were significantly higher than those of 2C acclimated animals, and lower, but not significantly different from those of animals acclimated at 18C. In May, values for animals acclimated at 10C approximated those of 2C acclimated animals.

Q_{10} values were calculated from mean rates of oxygen consumption determined in January, March, and May and are given in Table 1. These values were always less than two and decreased as the temperatures being compared increased, again except for 10C acclimated animals tested in May. Assuming that prior to acclimation all rates of oxygen uptake were equivalent to those of 18C acclimated animals, compensation in rates

Figure 2. Average rates of oxygen consumption of ammocoetes acclimated at 2, 10, and 18C measured in January, March, and May. Oxygen consumption is symbolized by Δ, ●, and O for animals acclimated at 2, 10, and 18C respectively. Vertical bars represent ± 1SE. Averages were calculated from values given in Appendix Tables 1, 2, and 3.

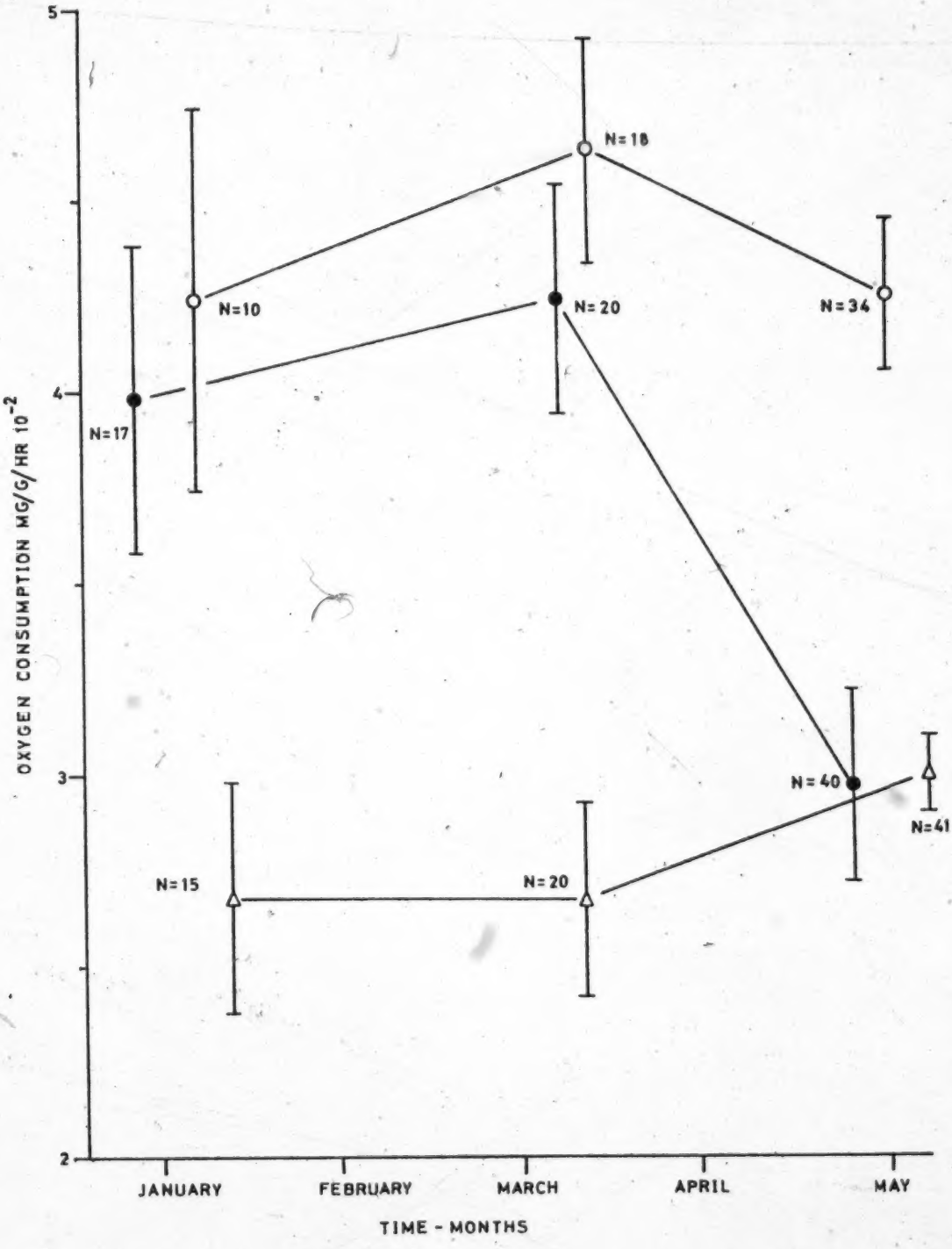

5
4
3
2
OXYGEN CONSUMPTION MG/G/HR 10^{-2}
N=18
N=10
N=20
N=34
N=17
N=40
N=41
N=15
N=20
JANUARY
FEBRUARY
MARCH
APRIL
MAY
TIME - MONTHS

TABLE 1. Long term Q_{10}*values calculated from average rates of oxygen consumption of ammocoetes acclimated at 2, 10, and 18C.

	Q_{10}		
	2-10C	10-18C	2-18C
January	1.64	1.08	1.33
March	1.74	1.12	1.39
May	0.98	1.56	1.24

*$Q_{10} = \frac{K_2}{K_1}^{\frac{10}{T_2-T_1}}$

where K_2 = the rate of oxygen uptake at temperature T_2
and K_1 = the rate at temperature T_1

of oxygen uptake were 58-75% after acclimation at 2C and 70-90% after acclimation at 10C.

B. Seasonal Variation of Oxygen Consumption, Body Weight and Length of Marked Ammocoetes

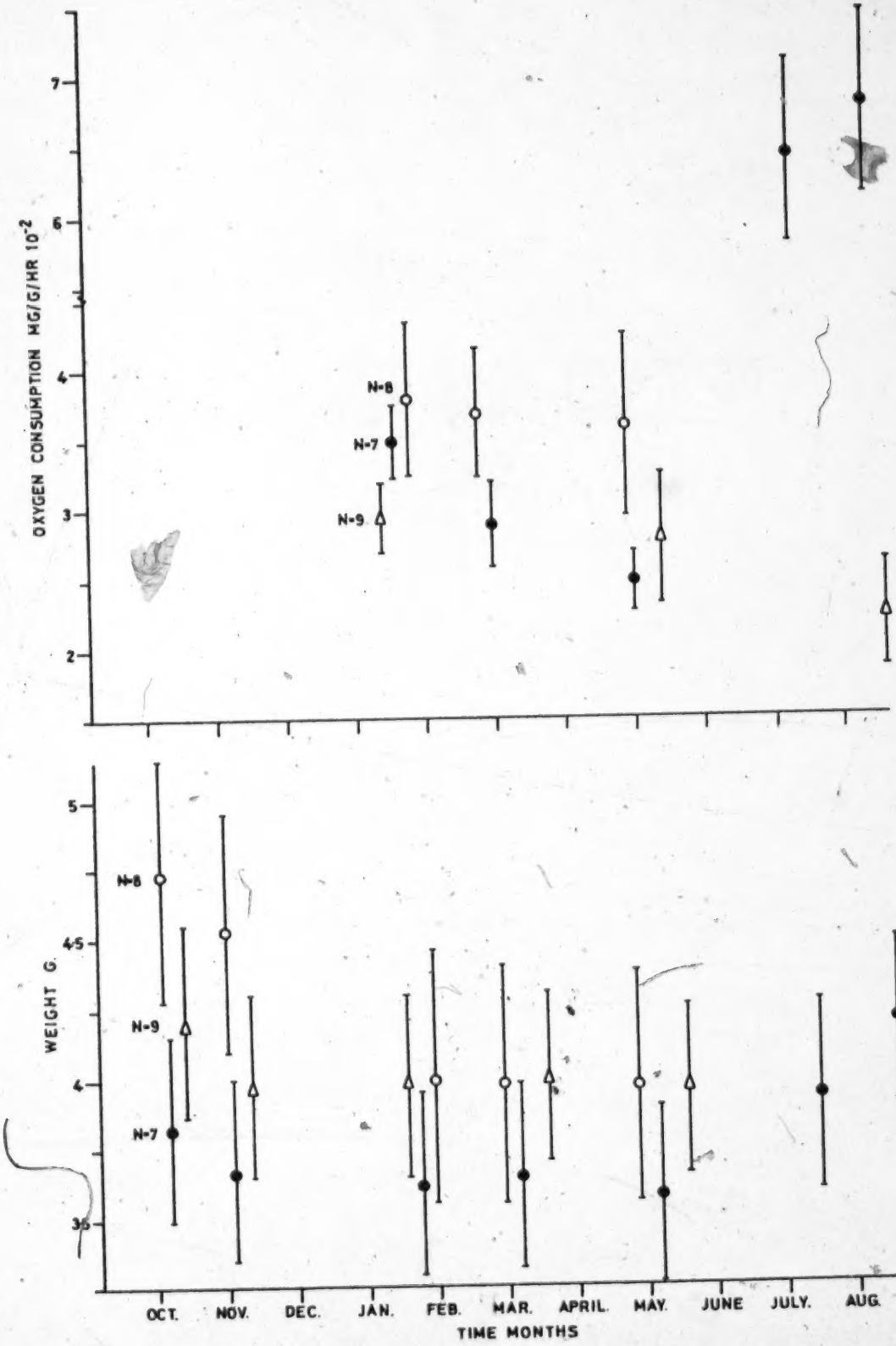
1. Oxygen consumption

Average rates of oxygen uptake determined at various times from January to August 1969 for marked ammocoetes acclimated at 2, 10, and 18C are given in Figure 3. At all temperatures, the average rate of oxygen uptake for marked animals decreased slightly from January to May, however only the difference between January and May values for 10C acclimated animals was significant. After May, rates of oxygen uptake continued to decrease slightly for animals at 2C, but increased sharply for animals acclimated at 10C; unfortunately, no data is available after May for animals acclimated at 18C.

Average rates of oxygen consumption of marked ammocoetes were generally within one standard deviation below the mean rates for unmarked animals when determined the same month (Figure 2). However in March, the average rate for marked 10C acclimated animals was significantly lower than that of the unmarked sample and approximated values of 2C acclimated animals, whereas oxygen uptake of the unmarked animals did not decrease this low until May.

Figure 3. Seasonal variation of oxygen consumption of marked ammocoetes acclimated at 2, 10, and 18C. Mean values are symbolized as in Figure 2 and were calculated from values given in Appendix Tables 4, 5, and 6.

Figure 4. Seasonal variation of body weight of marked ammocoetes acclimated at 2, 10, and 18C. Mean values are symbolized by Δ, ● and ○ for animals acclimated at 2, 10, and 18C respectively. Vertical bars represent ± 1SE. Averages were calculated from values given in Appendix Tables 4, 5, and 6.


OXYGEN CONSUMPTION MG/G/HR 10⁻²
7
6
4
3
2
N-8
N-7
N-9
WEIGHT G.
5
4·5
4
3·5
N-8
N-9
N-7
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.
APRIL.
MAY.
JUNE
JULY.
AUG.
TIME MONTHS

2. Body weight and length

Between October and November 1968, the average body weight of marked animals decreased significantly at all temperatures (Figure 4). In December, powdered fish food was replaced by powdered brine shrimp as a nutrient source and body weight at all temperatures stabilized and remained constant until the end of May. Between June and the end of August 1969, the average body weight of animals acclimated at 2C increased very slightly while that of animals acclimated at 10C increased sharply, surpassing the original October average weight.

Body lengths fluctuated slightly between October 1968 and May 1969 but there was no pattern to these changes (Appendix Tables 4, 5 and 6). However, from the end of May to the end of July, the average body length of 10C acclimated animals increased significantly 7mm; it then remained constant from July to August although body weight continued to increase. At 2C the average body length decreased 4mm between June and August even though body weight increased slightly.

C. Relationship Between Body Weight and Oxygen Consumption

Regression lines were determined for logarithmic plots of body weight versus both total oxygen uptake (mg/hr; Figure 5), and oxygen uptake per unit weight (mg/g/hr; Figure 6), for measurements made in January, March, and May on ammocoetes acclimated at 2, 10 and 18C. Both ways of representing oxygen consumption as a function of body weight have been included here since both are used in the literature.

Figure 5. Regression of body weight versus total oxygen uptake (mg/hr) of ammocoetes acclimated at 2, 10, and 18C determined in January, March, and May. Off scale values are symbolized by +. Correlation coefficients for each regression are given in the lower left corner of each graph.

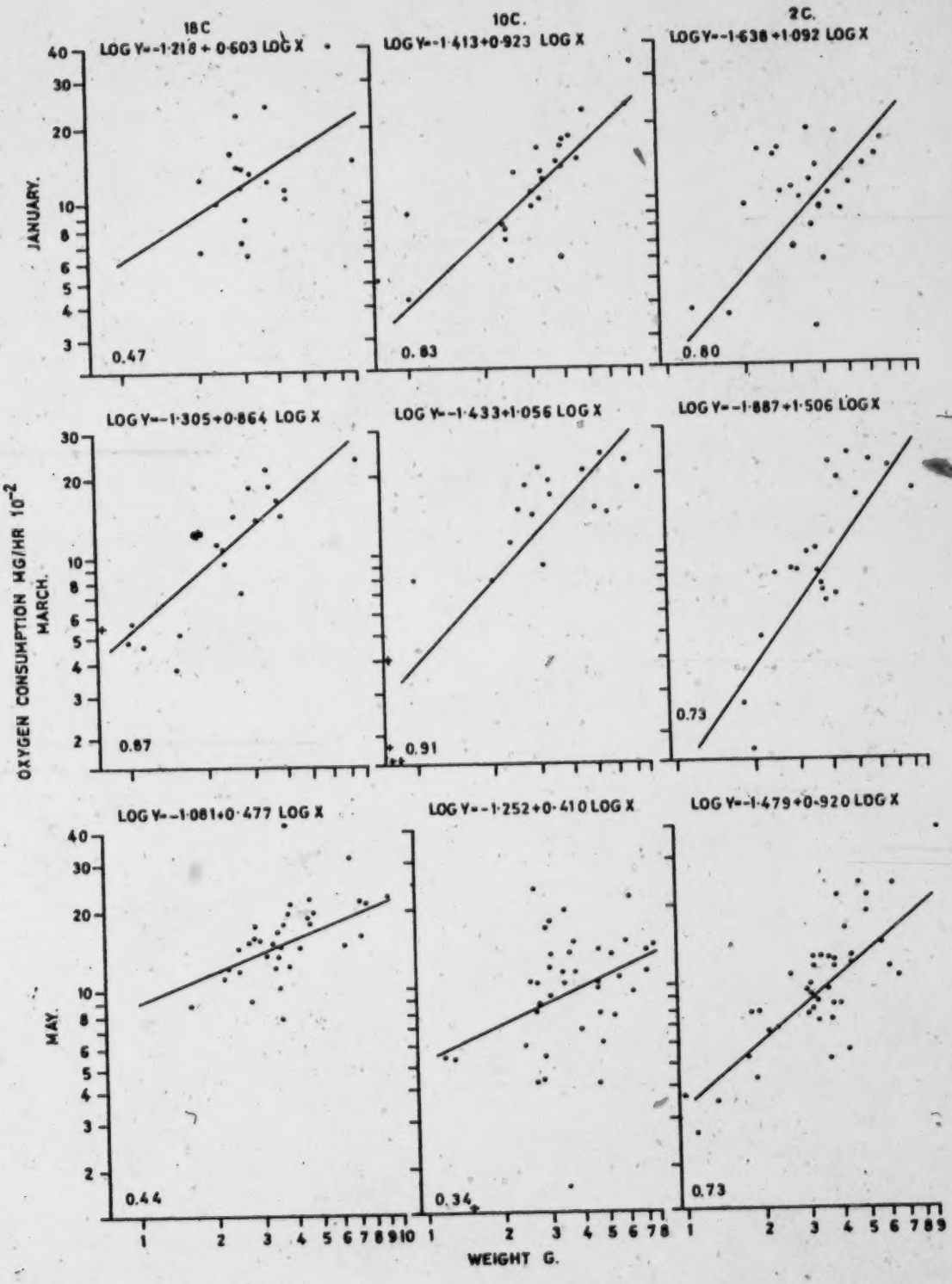
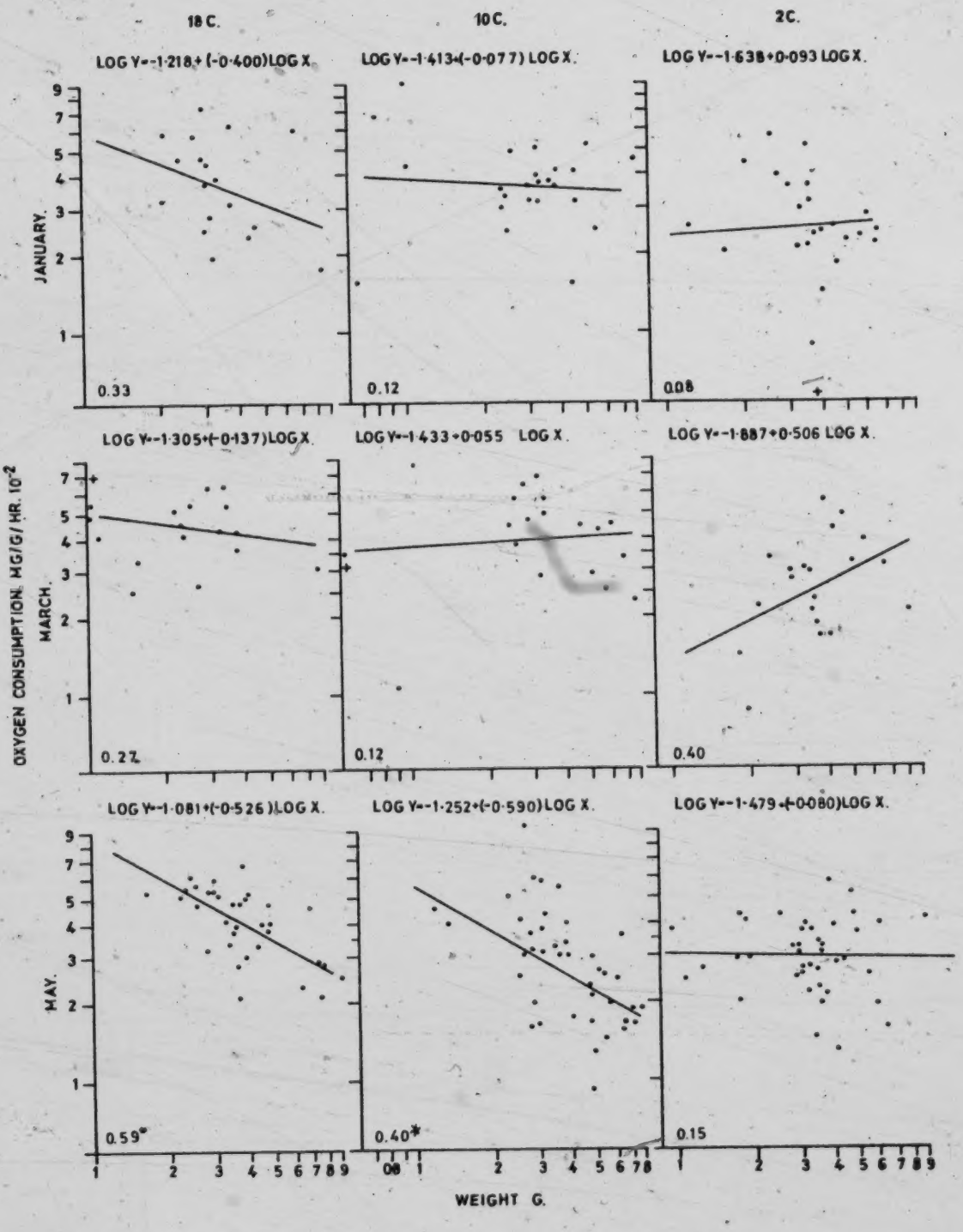

18C
LOG Y=-1·218 + 0·603 LOG X
10C.
LOG Y=-1·413+0·923 LOG X
2C.
LOG Y=-1·638 +1·092 LOG X
JANUARY.
0.47
0.83
0.80
LOG Y=-1·305+0·864 LOG X
LOG Y=-1·433+1·056 LOG X
LOG Y=-1·887+1·506 LOG X
OXYGEN CONSUMPTION MG/HR 10^{-2}
MARCH.
0.87
0.91
0.73
LOG Y=-1·081+0·477 LOG X
LOG Y=-1·252+0·410 LOG X
LOG Y=-1·479+0·920 LOG X
MAY.
0.44
0.34
0.73
WEIGHT G.

Figure 6. Regression of body weight versus oxygen uptake per unit weight of ammocoetes acclimated at 2, 10, and 18C determined in January, March, and May. Off scale values are symbolized by +. Correlation coefficients for each regression are given in the lower left corner of each graph. A * beside the correlation coefficient denotes significance ($P < 0.05$).


18 C.
10 C.
2 C.
LOG Y=-1·218+(-0·400)LOG X.
LOG Y=-1·413+(-0·077) LOG X.
LOG Y=-1·638+0·093 LOG X.
JANUARY.
0.33
0.12
0.08
LOG Y=-1·305+(-0·137)LOG X.
LOG Y=-1·433+0·055 LOG X.
LOG Y=-1·887+0·506 LOG X.
OXYGEN CONSUMPTION. MG/G/HR. 10^{-2}
MARCH.
0.27
0.12
0.40
LOG Y=-1·081+(-0·526)LOG X.
LOG Y=-1·252+(-0·590)LOG X.
LOG Y=-1·479+(-0·080)LOG X.
MAY.
0.59*
0.40*
0.15
WEIGHT G.

The best fit regression equation[12] for both relationships is log Y = a + b log X, where Y is oxygen uptake (mg/hr or mg/g/hr), a is the Y intercept, b the regression coefficient, and X the body weight. Regression equations and correlation coefficients for each acclimation temperature each month are given in Figures 5 and 6. Regression slopes were tested for significant differences using Student's t test (Steel and Torrie, 1960).

In calculating regression equations, the small sample size of unmarked animals tested in January was bolstered with values for marked animals tested the same month because regression coefficients are highly dependent on the size range of animals used, and the degree of confidence increases with sample size. However, to maintain statistical randomness in samples so that regressions could be compared between months, values for marked animals were not used to calculate March and May regressions.

Two patterns of change can be discerned in the slopes of the regression lines relating total oxygen uptake to body weight (Figure 5). The first is a decrease in the slope of the regression line with an increase in acclimation temperature. The regression coefficient of animals acclimated at 18C was significantly lower than that of animals acclimated at 2C all months tested. The regression coefficient of animals acclimated at 10C lay between values at 18 and 2C but was not significantly different from either except in May when it was significantly lower than values for 2C acclimated animals. Thus, as acclimation

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[12]Determined by use of the General Electric computer program SIXCUR $*** whereby data is fitted to six regression equations.

temperature increases, the increase in total oxygen uptake is greater for smaller animals than for larger ones.

The second pattern of change in the regression slopes relating total oxygen uptake to body weight is seasonal. Although the correlation between body weight and total oxygen uptake at each temperature was significant each month tested, regression coefficients increased between January and March then decreased significantly in May (Figure 5). May regression coefficients, although lower than January values at the equivalent temperature, were only significantly different for 10C acclimated animals.

Similar patterns of change between acclimation temperatures and between months can also be seen in the regressions relating body weight to oxygen uptake per unit weight (Figure 6). As acclimation temperature increased, the slope of the regression increased negatively all months tested. Seasonally, the regression coefficients at equivalent temperatures increased positively between January and March then decreased negatively in May. However, the correlation between body weight and oxygen uptake per unit weight was not significant except in May for animals acclimated at 18 and 10C. Thus, in May when the regression between total oxygen uptake and weight was lowest, the correlation between weight and oxygen uptake per unit weight was highest and significant for animals at 18 and 10C. At 2C, body weight was never significantly correlated with oxygen uptake per unit weight.

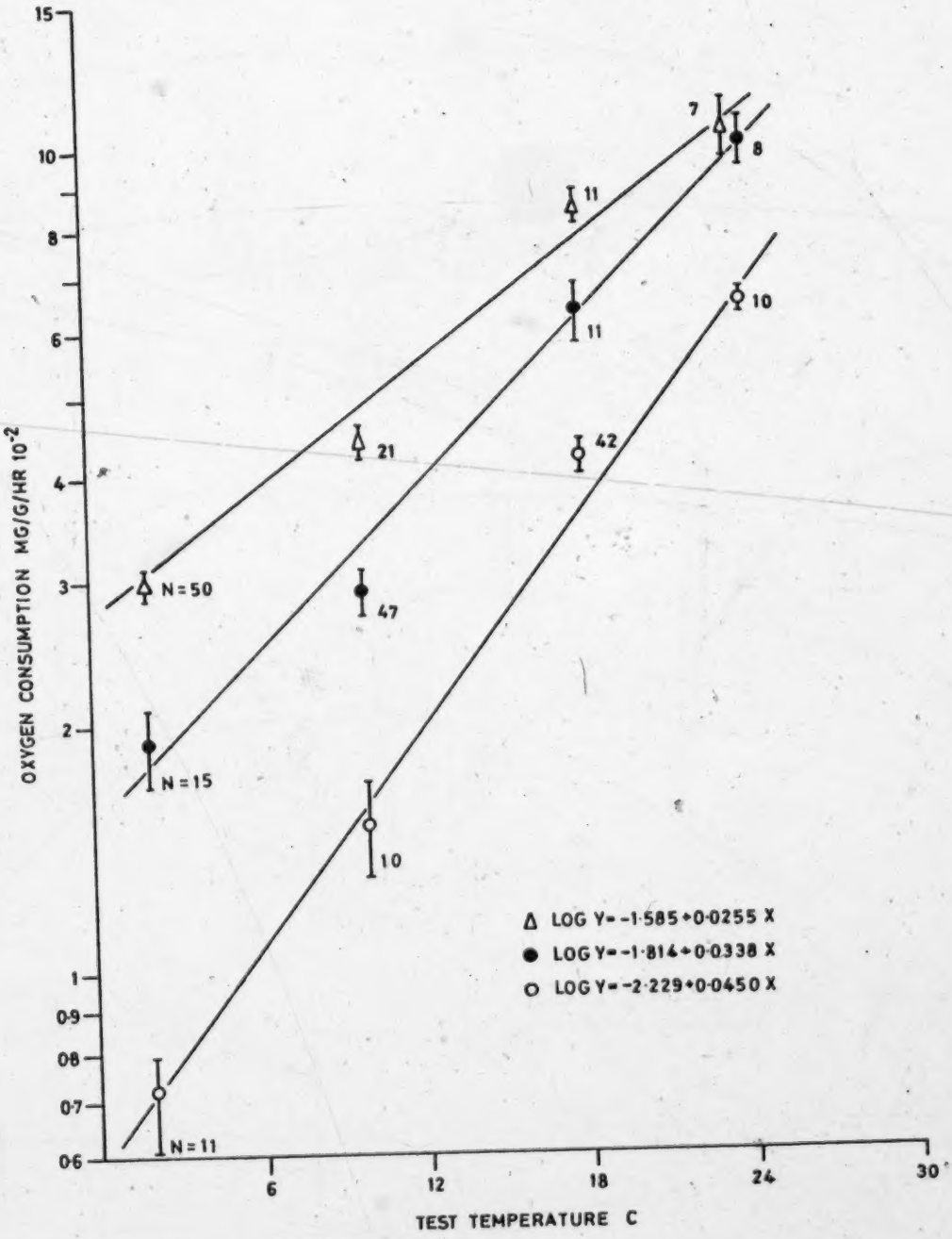
There was no significant difference in average body weight of animals measured at different temperatures or times of the year except at 18C where animals tested in March were significantly smaller than those tested in May.

D. Effect on Oxygen Consumption of Short Term Exposure to Temperatures Other than the Acclimation Temperature

Regression lines were calculated for semi-logarithmic plots of oxygen consumption per unit weight versus test temperature for 2, 10 and 18C acclimated animals tested at various temperatures during May and early June (Figure 7). The best fit regression equation is $\log Y = a + bX$, where Y is the rate of oxygen uptake (mg/g/hr), a is the Y intercept, b the regression coefficient, and X the test temperature. Regression slopes were tested for significant differences using Student's t test (Steel and Torrie, 1960).

As acclimation temperature decreased, the slope of the regression relating oxygen consumption to test temperature decreased. Using the terminology of Prosser (1961), this pattern of change in the regression slope is a Type IVA adaption wherein the regression is rotated clockwise and translated to the left as acclimation temperature decreases. The regression coefficient for animals acclimated at 2C was significantly lower than that of animals acclimated at 18C when $P < 0.02$, while that of animals acclimated at 10C was significantly lower than that of 18C acclimated animals when $P < 0.06$, and significantly higher than that of 2C acclimated animals when $P < 0.10$. Thus when $P < 0.10$, regression slopes for 2, 10, and 18C acclimated animals were all significantly different even though at the same level of significance, the mean rate of oxygen uptake of 10C acclimated animals tested at 10C was not significantly different from that of 2C acclimated animals tested at 2C.

Figure 7. Oxygen consumption of ammocoetes acclimated at 2, 10, and 18C measured at various test temperatures. Mean values are symbolized by Δ, ●, and ○ for animals acclimated to 2, 10, and 18C respectively. Vertical bars represent ± 1SE. Averages were calculated from values given in Appendix Table 7.


OXYGEN CONSUMPTION MG/G/HR 10⁻²
15
10
8
6
4
3
2
1
0·9
0·8
0·7
0·6
7
8
11
11
10
21
42
N=50
47
N=15
10
N=11
Δ LOG Y=-1·585+0·0255 X
● LOG Y=-1·814+0·0338 X
○ LOG Y=-2·229+0·0450 X
6
12
18
24
30
TEST TEMPERATURE C

The "acute" Q_{10} values for the regressions of test temperature versus rate of oxygen consumption are 1.77, 2.18, and 2.82 for animals acclimated at 2, 10, and 18C respectively. For the range of test temperatures used, the correlation between test temperature and the rate of oxygen consumption was highly significant and positive ($r = 0.99$) for each group of acclimated animals. No correlation could be determined between Q_{10} and body weight on the basis of the small sample sizes tested.

E. Effect of Acclimation Temperature on Hematocrit, Hemoglobin Concentration, and Oxygen Equilibria

Measurements made in late August 1969 on blood from ammocoetes acclimated at 2 and 10C (Tables 2 and 3) show no significant differences between hematocrits or hemoglobin concentrations of animals acclimated at different temperatures, even though the average rate of oxygen uptake of 10C acclimated animals was three times that of animals acclimated at 2C in August (Figure 3). Animals acclimated at 10C whose blood was used for these measurements were significantly heavier than 2C animals; however, with the sample size used, no correlation between body weight and hematocrit or hemoglobin concentration could be demonstrated.

Oxygen equilibria of hemoglobin solutions at different pHs are shown in Figures 8 and 9 for animals acclimated at 2 and 10C respectively. Hemoglobin solutions from ammocoetes acclimated at these temperatures had a very high affinity for oxygen when measured at 22C since P_{50}

TABLE 2. Hematocrits and hemoglobin concentrations of blood from ammocoetes acclimated at 2C

Hematocrit %	Hemoglobin g%	Body weight g
34.0	8.54	4.78
32.6	8.54	2.65
33.1	9.54	3.26
34.2	9.94	3.20
35.7	8.73	3.44
27.1	6.40	3.87
33.3	9.12	3.17
41.9	11.61	3.57
35.6	8.69	3.10
32.7	8.69	2.94
28.4	9.20	3.51
$\bar{X}$ 33.5	9.00	3.40
$\pm$1SD 4.0	1.24	
N 11		

TABLE 3. Hematocrits and hemoglobin concentrations of blood from ammocoetes acclimated at 10C

Hematocrit %	Hemoglobin g %	Body weight g
33.2	8.69	7.13
26.2	5.62	2.61
38.2	11.94	4.93
34.5	9.89	3.97
34.0	9.51	3.50
33.4	10.28	3.58
34.0	10.55	3.85
38.3	11.47	6.65
$\bar{X}$ 34.0	9.74	4.52
$\pm$1SD 3.8	1.96	
N 8		

Figure 8. Oxygen equilibria of hemoglobin solutions from ammocoetes acclimated at 2C, measured at pH 6.00, 6.47, and 7.15.

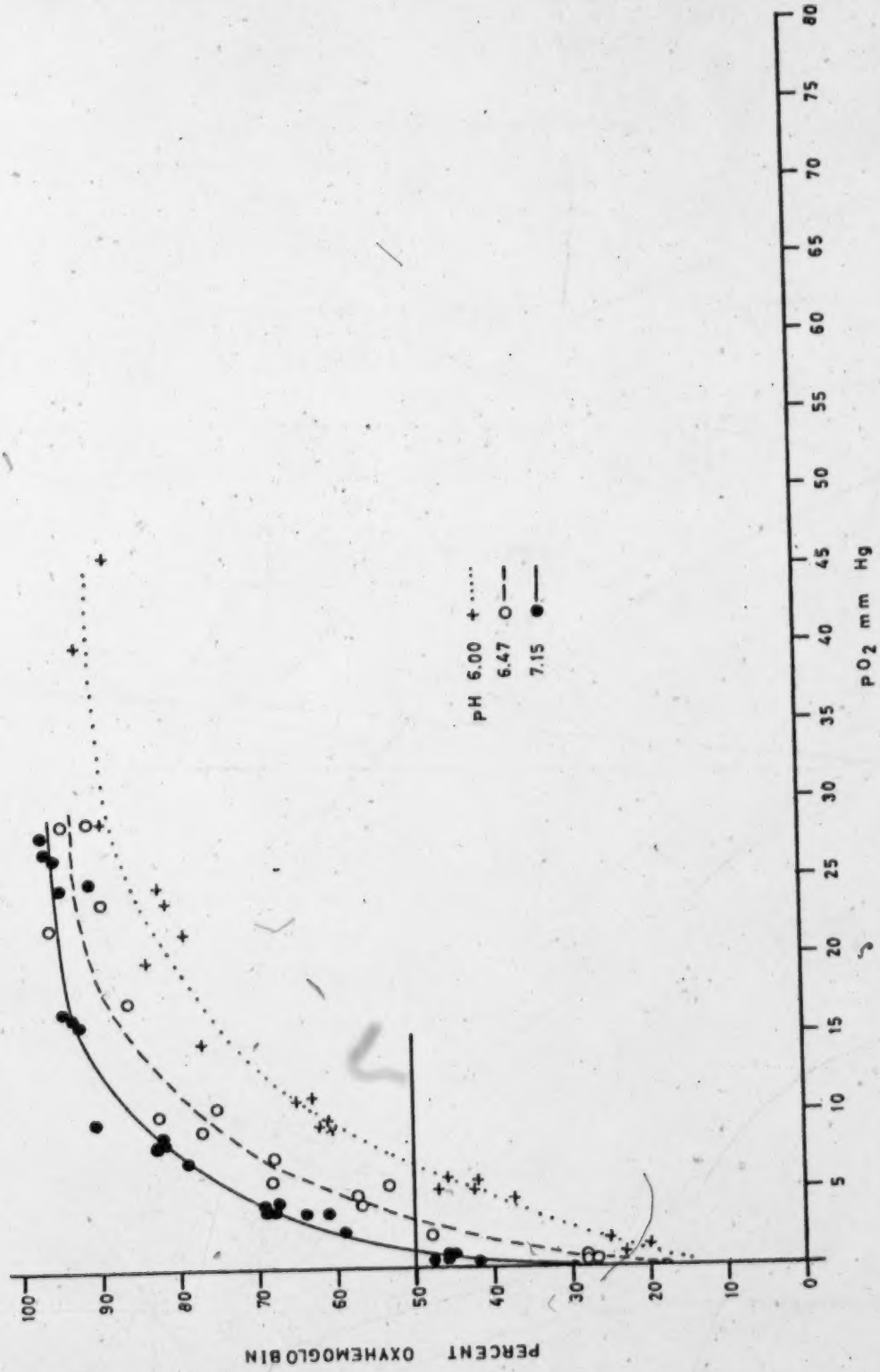
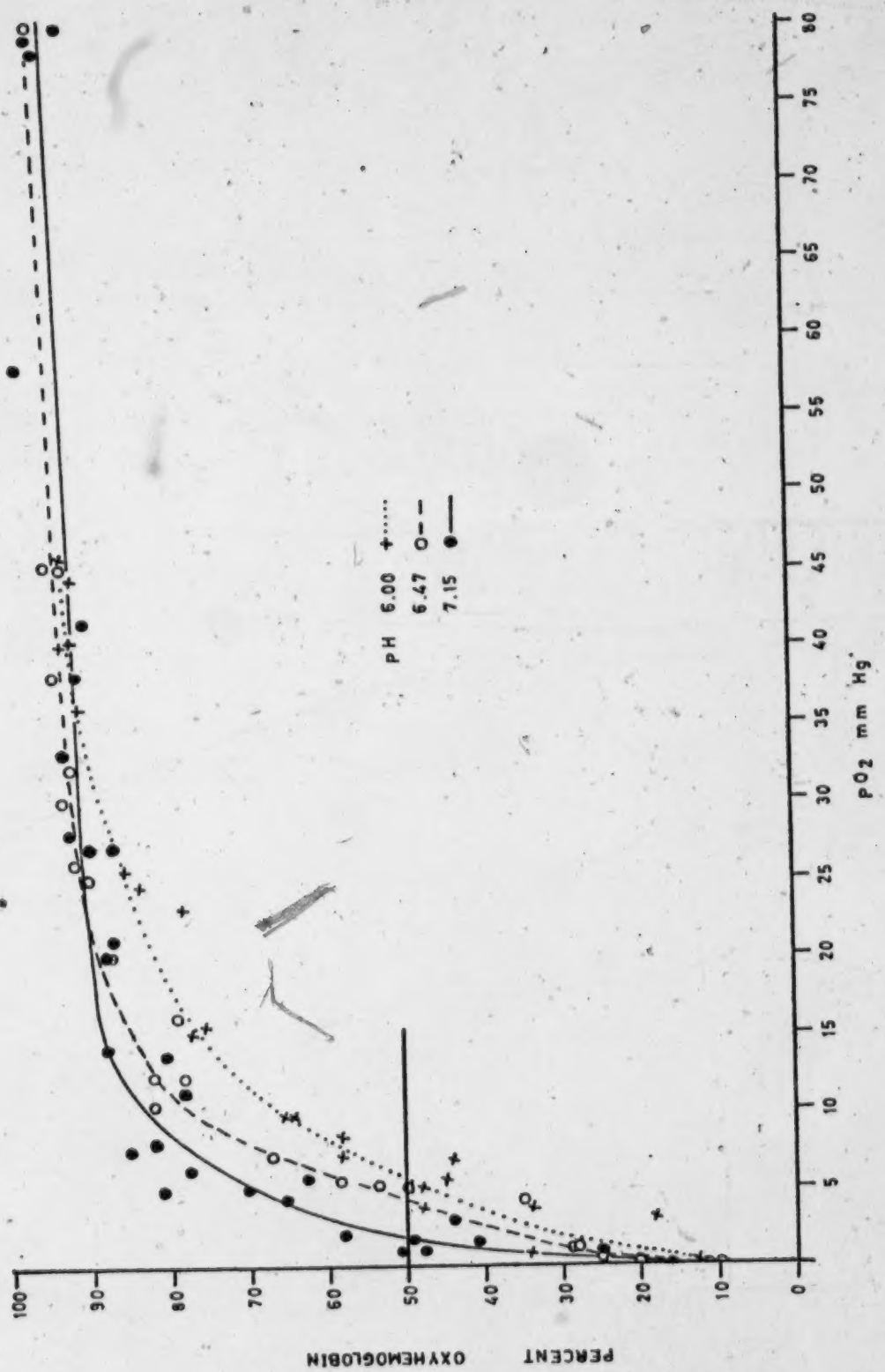

pH 6.00 +.....
6.47 O——
7.15 ●——
pO2 mm Hg
PERCENT OXYHEMOGLOBIN

Figure 9. Oxygen equilibria of hemoglobin solutions from ammocoetes acclimated at 10C, measured at pH 6.00, 6.47 and 7.15.


PERCENT OXYHEMOGLOBIN
pO2 mm Hg
pH 6.00
6.47
7.15

was less than 7 mmHg at all pHs tested. At pH 7.15, the most physiologically realistic pH, P_{50} was 1.5 mmHg for animals acclimated at both temperatures. Both temperature groups showed a similar decrease in oxygen affinity (increase in P_{50}) as the pH of the hemoglobin solution decreased. There was no appreciable difference between oxygen equilibria of the different temperature groups at each pH since P_{50} and P_{75} values were similar. However, P_{95} values of hemoglobin solutions from animals at 10C were higher than those of 2C acclimated animals at pH 7.15 and 6.47, but because of the small number of measurements taken at the higher partial pressures of oxygen, this difference cannot be considered significant.

Micro-pH equipment was not available to determine the actual pH of blood from each ammocoete, and there were not enough animals to enable pooling blood at each temperature for conventional pH measurements. However an excess of animals at 2C allowed for one pooled pH measurement - 7.42.

DISCUSSION

Fry (1971) argues that of the various methods using oxygen uptake as an indication of metabolic rate, only "standard" measurements calculated from "active" measurements of metabolic rate by extrapolation to either zero water turbulence (Beamish and Mookherjii, 1964) or zero swimming speed (Wohlschlag, 1957; Brett, 1964; Smit, 1965) realistically indicate the effect of acclimation temperature on the metabolic rate of fishes. The more commonly used "routine" measurements of metabolic rate determine the oxygen uptake of fish whose body movements, although minimized by either darkness or restricted space, are not eliminated, yet much of the change in metabolic rate which accompanies a change in temperature may be due to changes in the degree of locomotor activity (Wells, 1935; Roberts, 1960; Peterson and Anderson, 1969).

Unfortunately, measurements of active metabolic rate and thereby calculated standard metabolic rate are not feasible for larval lamprey whose normal habit is to remain burrowed and relatively inactive in the locomotor sense. When first placed in a container devoid of anything to hide or burrow in, ammocoetes swim immediately to the bottom of the container and attempt to burrow; within a minute they seem to exhaust themselves and lie quietly on the bottom. After a few minutes of rest they again attempt to burrow into the bottom of the container, exhaust themselves, then again rest. This is repeated two or three

times before they cease their burrowing attempts and lie quietly on the bottom unless disturbed by light or prodding (when exhausted, ammocoetes cannot be induced to swim by either light or prodding). Because activity seems to be "all or nothing" rather than degrees of mobility, the most realistic measurements of oxygen uptake in larval lamprey are those taken while the animals are burrowed either in a sterile natural substrate or in a suitable artificial substrate, such as the strands of plastic used in this study, or small glass beads used by Hill and Potter (1970) for larval *I. hubbsi*.

When larval *L. japonica* were placed in the respiration chambers, they immediately entwined in the plastic strands and movement ceased except for slight, very infrequent changes in position. The degree of immobility of the larvae once entwined in the strands can be illustrated by the fact that even when the chambers were inverted and the water drained, larvae rarely moved or attempted to escape. Therefore, although metabolic rate determined by this method is "routine" in that spontaneous activity is not eliminated, activity is so limited that these measurements come as close as possible to "standard" measurements where activity is nil.

The only problem with using an artificial substrate of plastic strands to quiet ammocoetes was that very small larvae (<1g) usually did not entwine in the strands and remained active during an experiment. Thus measurements from small larvae could only be used if no movement was observed.

In addition to questioning the use of routine measurements of oxygen uptake to indicate the degree of acclimation, some may question the use of closed respiration chambers to accurately measure oxygen uptake. Although no studies have been made on the effect of low oxygen or high carbon dioxide tensions on ammocoete oxygen uptake, Johansen et al. (1973) found that adult *E. tridentatus* were able to maintain a constant rate of oxygen uptake at water oxygen tensions above 35-40 mmHg at 20C, and 10 mmHg at 5 and 15C, partly by greatly increasing the breathing rate below water oxygen tensions of 100 mmHg. Observations made in July on burrowed *L. japonica* larvae held in 1 gallon jars and subjected to decreasing water oxygen tensions (created by bubbling in N_2 to decrease O_2 tensions by 1 ppm/hr) showed that the rate of velar beat remained constant above 30 mmHg at 10C [60-74 beats/min, N = 2], and 20 mmHg at 2C [18-25 beats/min, N = 2]. At water oxygen tensions of 7-10 mmHg, *L. japonica* emerged from the mud and swam vigourously near the surface of the water. Similarly, Potter et al. (1970) found that *I. hubbsi* larvae only emerged from the mud when oxygen tensions were lower than 10-12 mmHg at 15.5°C. As oxygen tension in the respiration chambers was never lower than 70 mmHg, it is unlikely that oxygen consumption of ammocoetes was affected by use of a closed system.

The method used in this study to determine oxygen consumption both at the temperature of acclimation and after short term exposure to various other "test" temperatures gives highly reproducible results. Mean weight-specific rates of oxygen consumption determined from May 13 to June 9, 1969 for four different groups of animals at each acclimation

temperature are surprisingly similar considering the range of individual oxygen uptakes at any one temperature (Appendix Table 7). After short term exposure to 10C, mean rates of oxygen uptake for two groups of animals acclimated at 2C and tested several days apart were 0.040 and 0.044 mg/g/hr, while two groups of animals acclimated at 10C and tested at 2C averaged 0.019 and 0.020 mg/g/hr. Test treatment and anaesthesia apparently did not affect subsequent test performance since the average rate of oxygen uptake for five marked larvae acclimated at 18C was 0.033 mg/g/hr when tested May 7 and 0.039 mg/g/hr when retested on May 10 (Appendix Table 6).

When mean rates of oxygen uptake are compared for different species of larval lamprey of similar size at equivalent temperatures and times of the year, values for *L. japonica* are in the same range as those for *I. hubbsi* (Hill and Potter, 1970), but much lower than values for *P. marinus* and *I. fossor* (Leach, 1946). However, values obtained by Leach (1946) are probably well above the standard rate since larvae were not provided with any substrate into which they could burrow. When larval *L. japonica* were tested in containers lacking a burrowing medium, rates of oxygen uptake were extremely variable and much higher than values presented in this thesis obtained when larvae were provided with plastic strands in which to burrow.

Unfortunately, Hill and Potter (1970) measured ammocoetes similar in size to *L. japonica* at only one acclimation temperature. The average rate of oxygen uptake they obtained - 0.055 mg/g/hr at 15.5C - is higher than the average value of 0.043 obtained for *L. japonica* acclimated at

18C measured at a similar time of year (January-March) (Figure 2). At acclimation temperatures of 3.5, 9.5 and 22.5C, Hill and Potter (1970) used smaller animals averaging 1.2 g. Mean rates of oxygen uptake obtained for these smaller *I. hubbsi* larvae at 3.5 and 9.5C (0.012 and 0.032 mg/g/hr) are lower than values for *L. japonica* at 2 and 10C respectively, and considering the inverse correlation between body weight and rate of oxygen uptake found for *I. hubbsi*, values at these lower temperatures would theoretically be even lower for animals of equivalent weight to *L. japonica*.

It is difficult to make relevant comparisons between larval and adult lamprey considering the great disparity in size and mode of life. Generally though, oxygen uptake in larval *L. japonica* is considerably lower than values recorded for adults. At 15C Sherbakov (1937) recorded an average value of 0.14 mg/g/hr for 37 g adult *L. fluviatilis*, while for adult *P. marinus* weighing 250-350 g, Beamish (1973) recorded an average rate of 0.108 mg/g/hr. At 18C the mean rate of oxygen uptake in *L. japonica* larvae was only 0.04 mg/g/hr. This is similar to the value of 0.049 mg/g/hr recorded for adult pre-spawning *E. tridentatus* at 15C, but lower than the value recorded at 20C of 0.129 mg/g/hr (Johansen et al., 1973). However, as Beamish (1973) points out, the low values recorded for *E. tridentatus* may reflect a deterioration in physiological condition known to accompany sexual development during the upstream spawning migration. Measurements of oxygen consumption for adult lamprey were standard for *P. marinus* (Beamish, 1973) but routine for *L. fluviatilis* (Sherbakov, 1937) and *E. tridentatus* (Johansen et al.,

1973). However, during measurement of oxygen uptake adults of all species clung to the sides of the chamber and rarely moved. Thus, as in larvae, routine measurements are close to standard rates where standard rates, calculated by extrapolation to zero activity as for *P. marinus*, include the cost of respiratory movement and the cost of clinging to the chamber wall. This latter cost may account for some of the difference between standard rates for adults and ammocoetes.

Oxygen consumption of larval *L. japonica* is much lower than routine measurements of oxygen uptake of teleosts at equivalent temperatures, even when size is similar (Winberg, 1956; Ralph and Everson, 1968; Hart, 1968). Some of the difference in routine measurements may be due to a greater degree of spontaneous activity in teleosts. However, even when activity is minimized or eliminated in some teleosts such as brook trout (Job, 1955; Beamish, 1964), brown trout [*Salmo trutta*] (Beamish, 1964), various species of Antarctic fish (Wohlschlag, 1964), yearling sockeye salmon (Brett, 1964), and underyearling Atlantic salmon (Peterson and Anderson, 1969), oxygen consumption is still higher than in larval *L. japonica* at equivalent temperatures, although the difference is greatly reduced and values for these teleosts approximate values for adult *P. marinus* and *L. fluviatilis*. Standard rates of oxygen consumption determined for the common white sucker [*Catostomus commersonii*] (Beamish, 1964) at 10C are equivalent to those for *L. japonica* at 10C, while values for the brown bullhead, carp (Beamish, 1964), goldfish (Beamish and Mookherjii, 1964), Pacific hagfish [*Eptatretus stoutii*] (Munz and Morris, 1965), and Australian lungfish [*Neoceratodus fosteri*] (Grigg, 1965) are lower than values for *L. japonica* at equivalent temperatures.

The Q_{10} value calculated from mean rates of oxygen uptake at the temperature of acclimation ("long-term" Q_{10}) was 1.3-1.4 for *L. japonica* over the temperature range of 2-18C and 1.5-1.7 between 2 and 10C (in January and March, Table 1), while in *I. hubbsi*, Q_{10} over the temperature range of 3.5-22.5C was 3.6, but between 3.5 and 9.5C, Q_{10} was 5.4. For both species Q_{10} values decreased as the temperatures being compared increased. The low Q_{10} values obtained for *L. japonica* larvae indicate that they are better equipped to adapt to a change in temperature than are *I. hubbsi* larvae. Although *L. japonica* is a parasitic species from the sub-Arctic zone while *I. hubbsi* is a non-parasitic species from the mid-temperate zone, both are subjected to similar temperature regimes in the natural environment. The difference in acclimation ability may be real but may in part be due to differences in maintenance of the animals prior to experimentation. *I. hubbsi* were collected in February and acclimated only nine days at 9.5C, two weeks at 3.5 and 22.5C and three weeks at 15.5C under constant light, whereas *L. japonica* were acclimated three months under a natural light cycle.

Although Hill and Potter (1970) found that *I. hubbsi* larvae did not exhibit a circadian rhythm in oxygen uptake, Kleerekoper, Taylor, and Wilton (1961) found that transforming and adult *P. marinus* did exhibit a circadian rhythm of activity which was eliminated when the animals were held under constant conditions of dim light. However, the relationship between circadian rhythms, photoperiod, and acclimation ability of fish has not been clearly defined. Hoar (1956) found that when goldfish were held at a constant temperature their thermal resistance could be

altered by exposure to different photoperiods, and Tyler (1966) has shown that at equal acclimation temperatures, redbelly dace were more resistant to high lethal temperatures in summer than in winter. Also, Evans, Purdie, and Hickman (1962) demonstrated that tissues of rainbow trout acclimated during the winter to 8L photoperiod tended to metabolize at a higher rate than tissues of fish acclimated to the 16L photoperiod. However, a similar effect could not be demonstrated for metabolic rates of intact trout. Roberts (1967) found that at temperatures above 10C the metabolic rate of sunfish acclimated to 9L was higher than that of fish acclimated to 15L at the same temperature, and that temperature compensation at 9L was almost complete over a higher temperature range (Q_{10} = 1.2 for 12.5-20C at 9L vs Q_{10} = 1 for 10-17.5C at 15L). Thus, under conditions of constant light, the acclimation ability of *I. hubbsi* larvae may not be the same as under natural light conditions.

Q_{10} values calculated for adult lamprey from mean rates of oxygen uptake determined at the acclimation temperature are only available for *P. marinus*. Over the temperature range of 5-20C, Q_{10} for *P. marinus* was 1.8, although between 10 and 15C, Q_{10} was 3.1 (Beamish, 1973). The average Q_{10} is comparable to that found for larval *L. japonica*, but the pattern of Q_{10} change with increasing temperature (i.e. a decrease in Q_{10} as the temperatures being compared increase) found for larvae was not apparent for adults. This pattern of Q_{10} change in larvae is similar however to that of some teleosts such as the brook trout (Job, 1955), brown trout, common white sucker, bullhead, carp (Beamish, 1964), and goldfish (Beamish and Mookherjii, 1964), and indicates that compensation

in standard oxygen uptake is more complete and the animals can more easily adapt to a change in temperature at higher temperatures. Depending on the rate of acclimation, this ability could be advantageous for larval lamprey since it would ensure a relatively stable metabolic rate during the warm summer months (approximately 10-20C) allowing consistent utilization of seasonal food sources and a steady rate of growth, providing of course that temperatures are not so high that all food energy is used for maintenance functions. The optimum and upper limiting temperatures for lamprey growth are not presently known. This also assumes that the acclimation ability of ammocoetes does not change seasonally but is similar or perhaps improved over that found for *L. japonica* in January and March. However, the sudden decrease in oxygen uptake of 10C acclimated ammocoetes in May followed by a sharp increase in both weight and oxygen uptake (Figures 2, 3 and 4) may have been accompanied by a change in acclimation ability in July and August but this was not measured. Oxygen uptake and weight did not vary during the summer for 2C acclimated animals, and unfortunately, measurements could not be made for 18C acclimated animals after May. However, the Q_{10} determined in May between 2 and 18C did not vary from March and January Q_{10}'s as it did between 10 and 2 or 18C.

Long term Q_{10} values calculated for *L. japonica* are lower than those recorded for most other teleosts (Job, 1955; Evans et al., 1962; Beamish, 1964; Beamish and Mookherjii, 1964; Brett, 1964; Rao, 1968; Hart, 1968; O'Hara, 1968), although within certain temperature limits the Q_{10} for sunfish is as low as that of *L. japonica* (Roberts, 1967).

Implicit here is that ammocoete *L. japonica* are better able to acclimate to changes in temperature. However, some of the difference in Q_{10} may reflect the more sedentary mode of life and inability to remain active for any length of time. Evans et al. (1962), Prosser (1967), and Precht (1968), indicate that muscle tissue in rainbow trout, goldfish, and eel [*Anguilla vulgaris*] respectively shows less compensation with temperature change than do gill and nervous tissues; however, muscle tissue of the bitterling [*Rhodeus amarus*] (Precht, 1968) and ide [*Idus indus*] (Berkholz, 1966) shows marked temperature compensation in oxygen uptake. Investigations into the acclimation ability of various tissue systems are as yet inconclusive as to which tissues are most important in acclimation of the total organism (Fry and Hochachka, 1970; Prosser, 1967).

Acute Q_{10} values calculated for larval *L. japonica* acclimated at different temperatures are similar to those found for most other fish wherein animals acclimated at cold temperatures have a low Q_{10} and high rate of oxygen consumption compared to warm acclimated animals (Precht, 1951; Bullock, 1955; Prosser and Brown, 1961; Morris, 1962; Grigg, 1965; Peterson and Anderson, 1969). Acute Q_{10} values for *L. japonica* are lower than those found for Atlantic salmon (Peterson and Anderson, 1969) but similar to values found for the more sedentary eel (Precht, 1951) and lungfish (Grigg, 1965). These Q_{10}'s based on standard or low routine rates of oxygen uptake are probably not comparable with Q_{10}'s based on routine rates of active species (Bullock, 1955; Prosser and Brown, 1961; Morris, 1962; 1965) since a change in temperature greatly alters activity (Peterson and Anderson, 1969). When ammocoetes acclimated

at 2C were transferred into water at 18 or 24C, swimming movements increased sharply, and instead of attempting to burrow, they sought to escape by leaping out of the water. Conversely, when animals acclimated at 18C were transferred to water at 2C, they sank to the bottom of the container and movements were few and extremely slow. However, when ammocoetes were entwined in plastic strands in a respiration chamber, a change in water temperature did not elicit movement.

Acute Q_{10} values have not been determined for other lamprey species acclimated at different temperatures, but for Pacific hagfish, Munz and Morris (1965) were unable to demonstrate any difference in acute Q_{10} or oxygen consumption at various test temperatures between fish acclimated at 4 or 10C. They attributed this to the thermally stable natural habitat of hagfish where such an acclimation ability would not offer a selective advantage. However, the peculiar experimental method of Munz and Morris whereby all animals regardless of acclimation temperature are held at the lowest test temperature overnight, then tested at progressively higher temperatures, may give different results from the experimental method used by others where animals are exposed to test temperatures for a short but equal period of time, and one individual is tested at only one temperature other than the acclimation temperature. Morris (1965) using the former method also found that acclimation temperature did not alter acute Q_{10} values in the yellow bullhead [*Ictalurus natalis*] although he was able to demonstrate that acute Q_{10} of the cichlid [*Aequidens portalegrensis*] did change with acclimation temperature, but only in larger fish (Morris, 1962).

Prosser (1963) has suggested that a change in acute Q_{10} with acclimation temperature implies "changes in the temperature characteristics" of enzymes "due to either an alteration in enzymes or in co-factors (qualitative changes or quantitative changes in relative amounts in parallel paths)", whereas a shift in the rate curve of oxygen uptake indicates "changes in the levels of enzyme activity." Although changes in enzyme concentration in poikilotherms have not been measured, changes in the kinds of enzymes present (Hochachka and Somero, 1968; Somero, 1969; Moon and Hochachka, 1971; Baldwin, 1971), and in enzyme activity probably through modulation of enzyme substrate affinity (Freed, 1965; Hochachka and Somero, 1969; Somero, 1969; Baldwin, 1971), as well as alterations in metabolic pathways (Hochachka and Hayes, 1962; Hochkachka, 1967) are known to occur in several poikilotherms when the body temperature is changed. However, the extent to which temperature induced enzyme changes account for changes in the Q_{10} or position of the curve relating oxygen consumption of the intact organism to test temperature has not been determined.

Although acute Q_{10} values have been shown to increase with increasing body weight (Rao and Bullock, 1954; Morris, 1962), this could not be demonstrated for *L. japonica* larvae. Similarly, Peterson and Anderson (1969) also found this relationship lacking in underyearling Atlantic salmon. Whether the absence of a correlation between body weight and acute Q_{10} is real or due to small sample size could only be determined by repeating the experiment using a larger sample size and wider size range of ammocoete.

Long term Q_{10} values, however, do show a definite weight effect since the slope of the line relating oxygen uptake (mg/hr) to body weight increased significantly between animals acclimated at 18 and 2C (Figure 5). Thus, the increase in oxygen consumption with an increase in acclimation temperature is greater in smaller ammocoetes, or, acclimation ability increases with size. Similar decreases in slope value with increasing acclimation temperature have been shown for brook trout (Job, 1955; Beamish, 1964), common white sucker, brown bullhead and carp (Beamish, 1964), but the differences were not significant with the sample sizes used. However, Evans et al. (1962) did find a significant increase in the slope for rainbow trout with decreasing acclimation temperature. O'Hara (1968) found that since the slope relating oxygen uptake to weight increased with acclimation temperature, large sunfish and blue gill [*Lepomis macrochirus*] were more affected by a change in temperature than were small fish. However he points out that the acclimation temperatures used were near the upper lethal limit and the same size - oxygen uptake relationship may not exist at lower temperatures. Hill and Potter (1970) did not determine the relationship between size and metabolic rate at different temperatures for *I. hubbsi* larvae, but for adult *P. marinus* Beamish (1973) found no temperature related change in slope values.

The regression of oxygen uptake (mg/hr) to body weight is generally described by values of 0.67 to 1.0 for fish (Bertalanffy, 1951; Winberg, 1956; Hickman, 1959; Beamish, 1964; Paloheimo and Dickie, 1966; Hill and Potter, 1970; Beamish, 1973). Slope values for ammocoetes were similar to those of other fish in January and March but in May, slope values for

animals acclimated at 18 and 10C decreased sharply to 0.427 and 0.448 respectively - much lower than values recorded for other fish.

Seasonally associated changes in slope values for *L. japonica* were evident at all temperatures. The increase from January to March in the slope of the regression at each temperature (Figure 5) was not significant but was primarily a result of either a limited size range of animals tested, or a few exceptionally high or low values of oxygen uptake distorting a regression based on a small sample size. In January, the size range of animals tested at 18C was very small - 14 animals weighing 2 to 4.5 g and two over 6 g. Variation in oxygen uptake of the 2 to 4 g animals was high, and the largest animal had an exceptionally low rate of oxygen uptake (Appendix Tables 1 and 6) - whereas in March at 18C, the size range sampled was evenly distributed between 0.7 to 4 g animals plus one 8 g animal, and the variation in oxygen uptake was much smaller than in January. At 10C, where the size ranges sampled in January and March were similar, the difference in the regression coefficient was not appreciable. At 2C, the increase in the regression slope in March was due primarily to exceptionally low rates of oxygen uptake for the only two animals smaller than 2 g and high rates for three of the 4 to 5 g animals which distorted the regression slope at either end. Since the regression coefficient is so easily altered by size range selection and sample size, a better estimate of the regression coefficient for the January-March period is obtained by pooling measurements. Pooled values yield regression coefficients of 0.76, 1.02 and 1.16 for animals acclimated at 18, 10 and 2C respectively. These are

significantly higher than slope values determined for the equivalent temperatures in May. The 18C value of the January-March period is comparable to a slope of 0.718 obtained for *I. hubbsi* larvae at 15.5C in February (Hill and Potter, 1970). Slope values for *L. japonica* at 18C are lower than for adult *P. marinus* at 15C (0.949) but values for ammocoetes at 10 and 2C measured in January and March are similar to those of *P. marinus* at equivalent temperatures (0.966, 0.933) measured in mid winter (Beamish, 1973).

The change in the slope relating oxygen uptake to body weight evident in May for *L. japonica* (Figure 5) was also reflected in a change in the slope relating oxygen uptake per unit weight to body weight for animals acclimated at 10 and 18C from an insignificant correlation to a significant correlation (Figure 6). Thus in May, but not January or March, oxygen consumption was dependent on body size at 10 and 18C but not at 2C. Similarly, Evans et al. (1962) found that the slope relating weight to oxygen uptake per unit weight was greater in rainbow trout acclimated at a longer photoperiod. The change in the relationship between oxygen uptake and body weight which occurred in May was followed by a significant increase in oxygen uptake and body weight of animals acclimated at 10C.

These changes in ammocoetes held at constant temperatures suggest the possibility of annual rhythms which are controlled by photoperiod and probably operate only under favourable temperature conditions. Because molecular activity is temperature limited, rates of oxygen consumption at 2C may be as high as possible at that temperature from January

to May and may not be capable of increasing as photoperiod increases. Moore and Beamish (1973) showed a marked decrease in digestive ability and injestion rate at 2C in larval *P. marinus*. However measurements at 2C were made in winter while measurements at higher temperatures were made in summer, therefore results may be photoperiod affected. Unfortunately data is not available for 18C acclimated animals after May, but I assume that oxygen uptake and growth would have increased, since the decrease in the slope of the line relating oxygen uptake (mg/hr) to body weight evident in May at 10 and 18C was followed by growth in July and August at 10C.

Most authors relate seasonal changes in oxygen consumption at a fixed temperature to changes in the reproductive state of the fish (Wohlschlag and Juliano, 1959; Evans et al., 1962; Roberts, 1967). However, ammocoetes are sexually immature and none showed signs of transformation or maturity to the end of October, 1969.

Seasonal changes in body size, oxygen consumption and the correlation between these two parameters measured in the laboratory environment can be compared with seasonal changes in the natural environment. During winter (0-2C), oxygen consumption is low, relatively stable and independent of body size (Figures 3, 5 and 6). Assuming there is sufficient food, body size is also stable (Figure 4) (Hill and Potter, 1970). In May, the ice and snow which blocked out nearly all light melts, and ammocoetes are suddenly exposed to long hours of light and rapidly increasing temperatures. As the food supply increases ammocoetes begin to grow (Hardisty, 1961) and oxygen consumption increases (Figure 3).

Since growth rate is greatest in young ammocoetes (Hardisty, 1961; Carlander, 1969), they consume more oxygen per unit weight than older, larger ammocoetes (Figure 6). Consequently the slope of the regression relating weight to oxygen consumption (mg/hr) decreases (Figure 5). (Unfortunately sample sizes were too small to determine a meaningful regression equation in July and August.) Similarly, Brett (1964) showed that juvenile salmon are characterized by a lower slope value (O_2mg/hr vs wt) than older salmon.

Open to question in suggesting the existence of annular rhythms based on the data in this thesis is the nutritional value of the food available to ammocoetes. The diet of larval *L. japonica* has not been determined, but the gut contents of American brook lampreys [*Lampetra lamottei*] (Creaser and Hann, 1929; Moore and Beamish, 1973), sea lamprey (Manion, 1967; Moore and Beamish, 1973), European brook lamprey, and Danube lamprey [*Eudontomyzon danfordi*] (Schroll, 1959) consist mainly of Aufwuchs, particularly the diatoms, in proportions similar to that found in the substrate. However it is not known to what extent each food item in the gut, including bacteria, detritus and dissolved nutrients, contributes to the nutritional requirements of lamprey. It is possible that powdered brine shrimp provided *L. japonica* was insufficient to allow for growth and that the rapid increase in weight at 10C in July was a result of a seasonal increase in the algal population. There was no visual evidence of an increase in the algal populations in any of the containers but no measurements were taken. To confirm the existence of annular rhythms, the experiments would have to be repeated and the diet controlled more closely.

Whatever the nature of the temperature or photoperiod induced changes in metabolism of ammocoetes, the adaptations are not reflected in hematocrits, hemoglobin concentrations or oxygen equilibria of hemoglobin solutions. Hematocrits and hemoglobin concentrations in larval *L. japonica* are higher than those found in *I. hubbsi* (Potter et al., 1970). Oxygen affinities of the hemoglobin solutions were very high and are comparable to *P. marinus* and *L. planerii* larvae and adult hemoglobin solutions at equivalent pH's and test temperatures (Manwell, 1963; Antonini et al., 1963), although higher than the affinity of intact red blood cells measured for *I. hubbsi* larvae (Potter et al., 1970). Measurements of oxygen affinity using red blood cells are likely more indicative of the situation in the intact organism (Riggs, 1972), particularly since oxygen equilibria presented in this thesis and by Manwell (1963) and Antonini et al. (1963) were measured over an unrealistic pH range, whereas Potter et al. measured oxygen equilibria of *I. hubbsi* blood at the actual pH of the blood.

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APPENDIX TABLE 1. Length, body weight, and oxygen consumption of ammocoetes acclimated at 2C, determined in January, March, and May 1969.

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
January		
76	0.57	0.0103
130	2.76	0.0396
133	2.61	0.0562
104	1.25	0.0298
120	1.74	0.0202
129	2.08	0.0445
151	3.68	0.0211
161	4.73	0.0187
164	4.58	0.0247
160	4.18	0.0241
153	4.04	0.0142
149	3.32	0.0298
184	6.78	0.0238
167	5.80	0.0226
180	6.41	0.0225
N 15	15	15
$\bar{X}$ 144	3.64	0.027
$\pm$1SD	1.63	0.012

continued . . .

APPENDIX TABLE 1 --continued

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
March		
131	2.15	0.0211
128	1.81	0.0139
145	2.96	0.0272
132	2.44	0.0320
129	1.95	0.0085
150	2.95	0.0275
156	3.78	0.0163
156	3.58	0.0225
156	3.94	0.0540
148	4.38	0.0417
155	4.73	0.0477
151	3.59	0.0202
155	3.67	0.0184
152	3.42	0.0287
152	4.07	0.016
148	3.28	0.0292
170	5.10	0.0310
174	5.71	0.0379
185	6.76	0.0300
197	8.21	0.0200
N 20	20	20
$\bar{X}$ 154	3.92	0.027
±1SD	1.56	0.011

Continued . . .

APPENDIX TABLE 1 - continued

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
May		
178	6.44	0.0157
160	5.90	0.0191
126	1.79	0.0422
154	3.13	0.0386
154	3.49	0.0263
147	3.23	0.0216
157	3.62	0.0223
154	3.28	0.0368
155	3.57	0.0196
99	0.95	0.0370
154	3.57	0.0328
153	3.83	0.0210
106	1.28	0.0269
107	1.08	0.0247
152	3.12	0.0266
131	2.51	0.0425
150	3.59	0.0313
152	3.00	0.0369
170	4.96	0.0364
156	4.22	0.0275
135	2.99	0.0247
146	3.02	0.0253
151	3.61	0.0322
140	2.91	0.0304
148	3.20	0.0267
154	3.82	0.0297
124	1.86	0.0230
152	3.42	0.0145

continued . . .

APPENDIX TABLE 1 - continued

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
May (continued)		
145	2.83	0.0320
123	1.88	0.0404
203	9.19	0.0405
188	6.03	0.0388
168	5.50	0.0253
170	4.96	0.0415
149	4.27	0.0283
156	4.04	0.0388
156	4.67	0.0506
123	1.72	0.0296
141	2.97	0.0319
151	4.12	0.0129
127	2.20	0.0302
N 41	41	41
$\bar{X}$ 148	3.56	0.030
$\pm$1SD	1.46	0.008

APPENDIX TABLE 2. Length, body weight, and oxygen consumption of ammocoetes acclimated at 10C, determined in January, March, and May 1969.

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
January		
85	0.65	0.0152
88	0.77	0.0654
99	1.01	0.0421
100	1.02	0.0905
141	2.63	0.0484
128	2.43	0.0293
136	2.52	0.0237
130	2.41	0.0326
155	3.27	0.0489
164	4.60	0.0314
154	3.07	0.0312
156	3.88	0.0356
156	3.28	0.0313
158	4.27	0.0409
160	4.24	0.0145
172	5.08	0.0504
190	7.80	0.0447
N 17	17	17
$\bar{X}$ 140	3.11	0.040
±1SD	1.62	0.018

continued . . .

APPENDIX TABLE 2 - continued

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
March		
102	0.88	0.0106
99	1.01	0.0754
81	0.55	0.0346
50	0.14	0.0306
125	2.44	0.0446
135	2.64	0.0382
143	2.79	0.0639
143	2.57	0.0564
148	2.90	0.0477
150	3.11	0.0681
153	3.34	0.0493
148	3.38	0.0554
167	4.67	0.0449
151	3.16	0.0283
172	5.01	0.0294
174	5.64	0.0249
173	5.42	0.0437
164	6.65	0.0331
177	6.02	0.0451
183	7.35	0.0232
N 20	20	20
$\bar{X}$ 142	3.48	0.042
$\pm$1SD	2.00	0.016

continued . . .

APPENDIX TABLE 2 - continued

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
May		
182	7.08	0.0160
166	4.64	0.0223
142	2.73	0.0199
102	1.30	0.0401
140	2.75	0.0158
151	3.69	0.0362
147	3.03	0.0576
163	3.84	0.0293
186	7.48	0.0186
175	6.08	0.0154
144	2.64	0.0906
145	2.65	0.0301
150	2.90	0.0586
170	4.66	0.0091
175	5.33	0.0143
153	3.48	0.0047
151	3.42	0.0326
151	3.03	0.0386
164	4.78	0.0126
185	5.97	0.0245
141	2.51	0.0404
159	3.96	0.0173
105	1.17	0.0450
171	5.02	0.0262
164	4.67	0.0165
176	5.49	0.0195
130	2.43	0.0245

continued . . .

APPENDIX TABLE 2 - continued

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
May (continued)		
146	3.00	0.0301
166	3.76	0.0395
142	2.71	0.0311
181	7.09	0.0187
175	6.13	0.0355
145	2.72	0.0372
175	5.25	0.0255
165	4.68	0.0209
169	4.67	0.0294
152	3.47	0.0555
159	3.49	0.0300
151	3.05	0.0429
145	2.75	0.0158
N 40	40	40
$\bar{X}$ 156	3.99	0.030
$\pm$1SD	1.44	0.016

APPENDIX TABLE 3. Length, body weight, and oxygen consumption of ammocoetes acclimated at 18C, determined in January, March, and May 1969.

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
January		
133	2.44	0.0426
140	2.99	0.0736
130	2.08	0.0320
130	2.12	0.0589
150	3.82	0.0622
141	3.14	0.0283
147	3.10	0.0378
143	3.09	0.0453
139	3.02	0.0239
189	8.27	0.0178
N 10	10	10
$\bar{X}$ 144	3.41	0.042
$\pm$1SD	1.53	0.017

continued . . .

APPENDIX TABLE 3 - continued

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
March		
86	0.72	0.0788
97	0.99	0.0491
100	1.04	0.0546
116	1.50	0.0250
116	1.58	0.0323
130	2.32	0.0410
133	2.58	0.0555
140	2.96	0.0625
138	2.68	0.0268
135	2.20	0.0515
106	1.13	0.0409
133	2.39	0.0458
149	3.19	0.0436
153	3.85	0.0368
145	3.51	0.0530
147	3.44	0.0630
152	3.79	0.0429
190	7.89	0.0304
N 18	18	18
$\bar{X}$ 125	2.65	0.046
±1SD	1.60	0.013

continued . . .

APPENDIX TABLE 3 - continued

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
May		
149	3.55	0.0285
142	2.81	0.0539
134	2.50	0.0478
151	3.70	0.0676
150	3.52	0.0479
135	2.20	0.0511
150	3.70	0.0476
140	3.03	0.0515
189	7.78	0.0272
139	2.29	0.0534
118	1.62	0.0538
143	2.82	0.0318
154	3.70	0.0210
203	9.34	0.0238
166	4.77	0.0376
171	6.85	0.0460
148	4.00	0.0527
169	4.91	0.0405
142	3.63	0.0401
150	3.53	0.0372
146	3.27	0.0413
135	2.50	0.0571
150	3.46	0.0347
134	2.43	0.0617
184	7.53	0.0212
187	7.52	0.0287
158	4.32	0.0338

continued . . .

APPENDIX TABLE 3 - continued

Length (mm)	Weight (g)	O_2 consumption (mg/g/hr)
May (continued)		
164	4.67	0.0400
149	2.96	0.0597
165	3.96	0.0304
179	6.34	0.0233
156	3.88	0.0510
142	2.91	0.0535
168	4.73	0.0471
N 34	34	34
$\bar{X}$ 155	4.14	0.042
±1SD	1.68	0.013

APPENDIX TABLE 4. Length, body weight, and oxygen consumption of marked ammocoetes acclimated at 2C.

	Length (mm)									
Ammocoete No.	1	2	3	4	5	6	7	8	9	$\bar{X}$
October 15, 1968	142	171	154	150	168	149	155	153	159	156
November 15, 1968	149	173	152	151	169	150	155	150	159	156
January 31, 1969	150	173	157	149	170	151	154	152	160	157
March 22, 1969	150	175	158	150	172	152	154	156	162	159
May 20, 1969	150	176	158	152	168	149	151	156	160	158
August 27, 1969	145	171	154	149	164	147	151	152	156	154

continued . . .

APPENDIX TABLE 4 - continued

Ammocoete No.	Weight (g) 1	2	3	4	5	6	7	8	9	$\bar{X} \pm 1SD$
October 15, 1968	3.00	6.40	3.70	3.90	5.40	3.30	4.10	3.80	4.20	4.20 ± 1.06
November 15, 1968	3.06	6.15	3.36	3.80	4.90	3.12	3.95	3.60	4.00	3.99 ± 0.98
January 31, 1969	3.02	6.07	3.50	3.76	5.03	3.12	3.86	3.62	3.94	3.99 ± 0.97
March 22, 1969	2.98	6.09	3.51	3.86	5.02	3.10	3.91	3.63	3.91	4.00 ± 0.98
May 26, 1969	2.98	6.02	3.51	3.86	4.87	3.06	3.86	3.72	4.00	3.99 ± 0.94
August 27, 1969	2.99	6.19	3.69	3.89	5.03	3.24	3.86	3.87	4.15	4.10 ± 0.97

[1] Oct. Nov. Jan. Mar. May Aug.

continued . . .

APPENDIX TABLE 4 - continued

	Oxygen consumption (mg/g/hr)									
Ammocoete No.	1	2	3	4	5	6	7	8	9	$\bar{K} \pm 1SD$
January 31, 1969	0.0362	0.0279	0.0519	0.0087	0.0224	0.0211	0.0351	0.0314	0.0231	0.029 ± 0.007
May 26, 1969	0.0560	0.0295	0.0252	0.0243	0.0169	0.0293	0.0327	0.0225	0.0182	0.028 ± 0.012
August 27, 1969	0.0108	0.0128	0.0227	0.0335	0.0405	0.0225	0.0133	0.0269	0.0198	0.022 ± 0.010

Jan. May Aug.

[1]Mean values for months underscored at the same level are not significantly different; values for months not underscored at the same level are significantly different ($P < 0.05$). Significant difference was determined by Student's paired t test (Steele and Torrie, 1960).

APPENDIX TABLE 5. Length, body weight and oxygen consumption of marked ammocoetes acclimated at 10C.

Length (mm)

Ammocoete No.	1	2	3	4	5	6	7	$\bar{X}$
October 15, 1968	162	175	154	144	135	147	149	152
November 13, 1968	162	175	154	146	138	147	150	153
February 1, 1969	159	174	154	143	134	146	149	151
March 16, 1969	162	176	160	145	135	149	150	154
May 13, 1969	160	176	162	147	136	149	149	154
July 26, 1969	173	181	168	154	143	156	152	161
August 25, 1969	175	178	165	152	145	157	155	161

Weight (g)

Ammocoete No.	1	2	3	4	5	6	7	$\bar{X} \pm 1SD$
October 15, 1968	4.00	5.75	4.09	3.26	2.50	3.49	3.65	3.82 ± 1.00
November 13, 1968	3.80	5.62	3.91	3.12	2.30	3.40	3.48	3.66 ± 1.01
February 1, 1969	3.75	5.56	3.92	3.07	2.38	3.36	3.34	3.62 ± 0.99
March 16, 1969	3.84	5.49	4.02	2.93	2.47	3.44	3.39	3.65 ± 0.96
May 13, 1969	3.70	5.34	3.95	2.97	2.50	3.28	3.30	3.58 ± 0.91
July 26, 1969	4.35	5.45	4.39	3.26	2.77	3.86	3.50	3.94 ± 0.88
August 25, 1969	4.65	5.57	4.56	3.43	3.16	4.01	4.08	4.21 ± 0.81
	Oct.	Nov.	Feb.	Mar.	May	July	Aug.	

continued . . .

APPENDIX TABLE 5 - continued

Ammocoete No.	Oxygen consumption (mg/g/hr) 1	2	3	4	5	6	7	X ± 1SD
February 1, 1969	0.0369	0.0243	0.0411	0.0357	0.0338	0.0362	0.0388	0.035 ± 0.005
March 16, 1969	0.0342	0.0192	0.0239	0.0357	0.0377	0.0271	0.0315	0.030 ± 0.007
May 13, 1969	0.0249	0.0302	0.0270	0.0266	0.0269	0.0221	0.0119	0.024 ± 0.004
July 26, 1969	0.0537	0.0491	0.0546	0.1006	0.0711	0.0588	0.0636	0.064 ± 0.018
August 25, 1969	0.0547	0.0712	0.0728	0.0759	0.1005	0.0501	0.0517	0.068 ± 0.018

Feb. Mar. May July Aug.

APPENDIX TABLE 6. Length, body weight, and oxygen consumption of marked ammocoetes acclimated at 18C.

	Length (mm)								
Ammocoete No.	1	2	3	4	5	6	7	8	$\bar{X}$
October 15, 1968	148	164	150	185	161	151	165	151	159
November 12, 1969	150	167	151	185	162	152	160	151	160
February 2, 1969	139	162	149	180	163	142	156	145	154
March 15, 1969	141	165	149	178	164	146	156	147	156
May 7, 1969	146	165	150	179	169	150	161	152	159
May 7, 1969		165	150	179	169	150	161		162
May 10, 1969		165	149	179	168	149	158		161

continued . . .

APPENDIX TABLE 6 - continued

Ammocoete No.	Weight (g) 1	2	3	4	5	6	7	8	$\bar{X} \pm 1SD$
October 15, 1968	3.75	4.30	4.15	7.55	5.10	3.52	5.40	4.50	4.78 ± 1.28
November 12, 1968	3.65	4.09	4.05	7.40	5.01	3.26	5.10	3.70	4.53 ± 1.32
February 2, 1969	2.98	3.84	3.29	6.77	4.59	2.76	4.55	3.29	4.01 ± 1.31
March 15, 1969	2.90	3.93	3.55	6.44	4.61	2.79	4.36	3.37	3.99 ± 1.18
May 7, 1969	2.94	4.03	3.23	6.35	4.57	2.89	4.39	3.33	3.96 ± 1.16
May 7, 1969		4.03	3.23	6.35	4.57	2.89	4.39		4.24 ± 1.22
May 10, 1969		3.96	3.27	6.34	4.73	2.96	4.32		4.26 ± 1.24

Oct.	Nov.	Feb.	Mar.	May
	May 7			May 10

continued . . .

APPENDIX TABLE 6 - continued

Ammocoete No.	Oxygen consumption (mg/g/hr) 1	2	3	4	5	6	7	8	$\bar{X} \pm 1SD$
February 2, 1969	0.0472	0.0319	0.0196	0.0599	0.0248	0.0572	0.0236	0.0397	0.038 ± 0.016
March 15, 1969	0.0532	0.0495	0.0285	0.0283	0.0387	0.0411	0.0185	0.0401	0.037 ± 0.012
May 7, 1969	0.0382	0.0393	0.0206	0.0171	0.0356	0.0498	0.0210	0.0688	0.036 ± 0.017
May 7, 1969		0.0393		0.0171	0.0356	0.0498	0.0210		0.033 ± 0.013
May 10, 1969		0.0304		0.0233	0.0471	0.0597	0.0338		0.039 ± 0.014

Feb. Mar. May

May 7 May 10

APPENDIX TABLE 7. Oxygen consumption and body weight of ammocoetes acclimated at 2, 10, and 18C, measured at various test temperatures.

Acclimation temperature:	2C		10C			18C		
Weight (g)	O_2 consumption (mg/g/hr) at 2C	24C	Weight (g)	O_2 consumption (mg/g/hr) at 10C	24C	Weight (g)	O_2 consumption (mg/g/hr) at 18C	24C
6.44	0.0157	0.0579	7.08	0.0160	0.0764	7.53	0.0212	0.0625
5.90	0.0191	0.0836	4.64	0.0223	0.0886	7.52	0.0287	0.0614
1.79	0.0422	0.1418	2.73	0.0199	0.0795	4.32	0.0338	0.0643
3.13	0.0386	0.1130	1.30	0.0401	0.1440	4.67	0.0400	0.0645
3.49	0.0263	0.0672	2.75	0.0158	0.1531	2.96	0.0597	0.0788
3.23	0.0216	0.1430	3.69	0.0362	0.1057	3.96	0.0304	0.0564
3.62	0.0223	0.1241	3.03	0.0576	0.1191	6.34	0.0233	0.0511
			3.84	0.0293	0.0555	3.88	0.0510	0.0594
						2.91	0.0535	0.0799
						4.73	0.0471	0.0632
N 7	7	7	8	8	8	10	10	10
$\bar{X}$ 3.94	0.026	0.104	3.63	0.030	0.103	4.88	0.039	0.064

	2C	18C		10C	18C		18C	18C
9.19	0.0405	0.0749	7.48	0.0186	0.0538	3.55	0.0285	0.0432
6.03	0.0388	0.0613	6.08	0.0154	0.0358	3.33	0.0688	0.0426*
5.50	0.0253	0.0743	2.64	0.0906	0.0882	6.35	0.0171	0.0120*
4.96	0.0415	0.0713	2.65	0.0301	0.0801	4.03	0.0393	0.0407*
4.27	0.0283	0.0849	2.90	0.0586	0.0758	4.37	0.0210	0.0301*
4.04	0.0388	0.0849	4.66	0.0091	0.0491	2.81	0.0539	0.0518
4.67	0.0506	0.0717	5.33	0.0143	0.0431	4.57	0.0356	0.0379*
1.72	0.0296	0.0967	3.48	0.0047	0.0609	2.89	0.0498	0.0520*
2.97	0.0319	0.1044	3.42	0.0326	0.0798	2.94	0.0382	0.0392*
4.12	0.0129	0.0924	3.03	0.0386	0.0723	3.23	0.0206	0.0540*
2.20	0.0302	0.1054	4.78	0.0126	0.0587			
N 11	11	11	11	11	11	10	10	10
$\bar{X}$ 4.52	0.034	0.084	4.22	0.030	0.063	3.80	0.037	0.040

continued . . .

APPENDIX TABLE 7 - continued

Acclimation temperature:	2C			10C			18C	
Weight (g)	O_2 consumption (mg/g/hr) at 2C	O_2 consumption (mg/g/hr) at 10C	Weight (g)	O_2 consumption (mg/g/hr) at 10C	O_2 consumption (mg/g/hr) at 10C	Weight (g)	O_2 consumption (mg/g/hr) at 18C	O_2 consumption (mg/g/hr) at 10C
4.96	0.0364	0.0564	5.97	0.0245	0.0265	2.50	0.0478	-
4.22	0.0275	0.0408	3.95	0.0270	0.0230*	3.70	0.0676	0.0215
2.99	0.0247	0.0584	5.34	0.0302	0.0319*	3.52	0.0479	0.0088
3.02	0.0253	0.0680	3.28	0.0505	0.0420	2.20	0.0511	0.0294
2.91	0.0304	0.0338	2.51	0.0404	0.0400	3.70	0.0476	0.0126
3.20	0.0268	0.0490	3.96	0.0173	0.0200	3.03	0.0515	0.0209
3.82	0.0297	0.0567	3.30	0.0119	0.0125*	7.78	0.0272	0.0022
1.86	0.0230	0.0356	3.70	0.0249	0.0306*	2.29	0.0534	0.0050
3.42	0.0145	0.0264	2.97	0.0266	0.273*	1.62	0.0538	0.0167
3.57	0.0196	0.0288	2.50	0.0269	0.280*	2.82	0.0318	0.0248
0.95	0.0380	0.0486				3.70	0.0210	0.0108
3.57	0.0328	0.0364						
3.83	0.0210	0.0387						
1.28	0.0269	0.0555						
3.12	0.0266	0.0327						
2.51	0.0425	0.0394						
3.54	0.0314	0.0356						
3.00	0.0369	0.0478						
3.61	0.0322							
3.28	0.0368							
1.08	0.0247							
N 21	21	18	10	10	10	11	11	10
$\bar{X}$ 3.04	0.029	0.044	3.75	0.028	0.028	3.35	0.046	0.015

continued . . .

APPENDIX TABLE 7 - continued

Acclimation temperature: 2C		10C			18C		
Weight (g)	O_2 consumption (mg/g/hr) at 2C	Weight (g)	O_2 consumption (mg/g/hr) at 10C	at 2C	Weight (g)	O_2 consumption (mg/g/hr) at 18C	at 2C
6.02	0.0295*	1.17	0.0450	0.0172	9.34	0.0238	0.0041
4.87	0.0169*	5.02	0.0262	0.0068	4.77	0.0376	0.0147
2.83	0.0320	4.67	0.0165	0.0122	6.85	0.0460	0.0126
3.06	0.0292*	5.49	0.0195	0.0070	4.00	0.0527	0.0057
4.00	0.0182*	2.43	0.0245	0.0379	4.91	0.0405	0.0051
3.72	0.0222*	3.00	0.0301	0.0278	3.63	0.0401	0.0054
1.88	0.0404	3.76	0.0395	0.0226	3.53	0.0372	0.0074
2.98	0.0560*	2.71	0.0311	-	3.27	0.0413	0.0043
3.86	0.0327*	7.09	0.0187	0.0112	2.50	0.0571	0.0053
3.51	0.0252*	6.13	0.0355	0.0113	3.46	0.0347	0.0063
3.86	0.0243*	2.72	0.0372	0.0367	2.44	0.0617	0.0086
		5.25	0.0255	0.0131			
		4.68	0.0209	0.0110			
		4.67	0.0294	0.0230			
		3.47	0.0555	0.0357			
		3.49	0.0300	0.0161			
		3.05	0.0429				
		2.75	0.0158				
N 11	11	18	18	15	11	11	11
$\bar{X}$ 3.69	0.030	3.98	0.030	0.019	4.43	0.043	0.007
Total							
N 50	50	47	47		42	42	
$\bar{X}$ 3.63	0.030	3.93	0.030		4.10	0.041	

*Marked ammocoetes